The New England Journal of Medicine

VOLUME 340 MAY 13, 1999 NUMBER 19

THE EFFECT OF FOLIC ACID FORTIFICATION ON PLASMA FOLATE AND TOTAL HOMOCYSTEINE CONCENTRATIONS

PAUL F. JACQUES, SC.D., JACOB SELHUB, PH.D., ANDREW G. BOSTOM, M.D., PETER W.F. WILSON, M.D., AND IRWIN H. ROSENBERG, M.D.

ABSTRACT

Background In 1996, the Food and Drug Administration issued a regulation requiring all enriched grain products to be fortified with folic acid to reduce the risk of neural-tube defects in newborns. Fortification (140 μg per 100 g) began in 1996, and the process was essentially complete by mid-1997.

Methods To assess the effect of folic acid fortification on folate status, we measured plasma folate and total homocysteine concentrations (a sensitive marker of folate status) using blood samples from the fifth examination (January 1991 to December 1994) of the Framingham Offspring Study cohort for baseline values and the sixth examination (January 1995 to August 1998) for follow-up values. We divided the cohort into two groups on the basis of the date of their follow-up examination: the study group consisted of 350 subjects who were seen after fortification (September 1997 to March 1998), and the control group consisted of 756 subjects who were seen before fortification (January 1995 to September 1996).

Results Among the subjects in the study group who did not use vitamin supplements, the mean folate concentrations increased from 4.6 to 10.0 ng per milliliter (11 to 23 nmol per liter) ($P<0.001$) from the baseline visit to the follow-up visit, and the prevalence of low folate concentrations (<3 ng per milliliter [7 nmol per liter]) decreased from 22.0 to 1.7 percent ($P<0.001$). The mean total homocysteine concentration decreased from 10.1 to 9.4 μmol per liter during this period ($P<0.001$), and the prevalence of high homocysteine concentrations (>13 μmol per liter) decreased from 18.7 to 9.8 percent ($P<0.001$). In the control group, there were no statistically significant changes in concentrations of folate or homocysteine.

Conclusions The fortification of enriched grain products with folic acid was associated with a substantial improvement in folate status in a population of middle-aged and older adults. (N Engl J Med 1999;340:1449-54.)

IN 1996, the Food and Drug Administration (FDA) issued a regulation, to be effective by January 1998, requiring that all enriched flour, rice, pasta, cornmeal, and other grain products contain 140 μg of folic acid per 100 g in addition to the thiamine, riboflavin, niacin, and iron already present in such products.[1] The goal of this folic acid fortification was to increase the intake of folate by women of childbearing age in response to the recommendation of the Public Health Service that "all women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected with spina bifida or other NTDs (neural tube defects)."[2] This recommendation followed the release of the results of a randomized, controlled clinical trial that found that vitamin supplements containing folic acid prevented many neural-tube defects.[3] This outcome was consistent with the results of other randomized trials,[4,5] nonrandomized trials,[6,7] and observational studies[8-11] of periconceptional folate intake. It was estimated that folic acid fortification at the level of 140 μg per 100 g would provide an additional 80 to 100 μg of folic acid per day to the diet of women of childbearing age and 70 to 120 μg to the diet of middle-aged and older adults.[12] Discussion continues regarding the need for a higher level of fortification.[13]

Data to assess the initial effect of folic acid fortification on plasma folate concentrations are available from a population-based sample of middle-aged and older adults who attended the fifth and sixth exam-

From the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston (P.F.J., J.S., I.H.R.); the Division of General Internal Medicine, Memorial Hospital of Rhode Island, Providence (A.G.B.); and the Framingham Heart Study, National Heart, Lung, and Blood Institute, Framingham, Mass. (P.W.F.W.). Address reprint requests to Dr. Rosenberg at the Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington St., Boston, MA 02111.

ination cycles of the Framingham Offspring Study. The fifth examination was completed before the implementation of fortification and provides data on prefortification folate status for all members of the Framingham Offspring cohort. The sixth examination was started before fortification but continued until after full implementation of fortification, thus providing a group of persons who were exposed to folic acid fortification and a comparable group who were not. We also used the plasma total homocysteine concentration, which is a sensitive functional marker of cellular folate status,[14] to assess the effect of fortification.

METHODS

Subjects

The Framingham Heart Study, an epidemiologic study of heart disease, was established in Framingham, Massachusetts, between 1948 and 1950 with a cohort of 5209 men and women who were 30 to 59 years of age.[15] By 1971, the original cohort included 1644 married couples and 378 individuals in whom cardiovascular disease had developed. The offspring of these subjects and the spouses of the offspring were invited to participate, and 5135 of the 6838 eligible persons participated in the first examination of the Framingham Offspring Study.[16] The offspring cohort has subsequently undergone follow-up examinations approximately every three to four years. The fifth examination of the offspring cohort began in January 1991 and was completed in December 1994. The sixth examination began in January 1995 and was completed in August 1998.

Determination of Exposure to Folic Acid Fortification

The final regulation for folic acid fortification of grain products was issued in March 1996, with an effective date of January 1, 1998. The FDA established this two-year period to allow manufacturers to exhaust packaging inventory and to update labels. However, the FDA stated that compliance could begin immediately.[1] To our knowledge there was minimal, if any, fortification of foods before September 1996. In New England, most of the targeted products were fortified with folic acid by July 1997 (Watson J, Watson Foods, New Haven, Conn.: personal communication).

The fifth examination of the cohort was completed before fortification began. The sixth examination began before the start of fortification and continued until after fortification was in place. We identified members of the cohort whose sixth examination occurred after targeted foods began to be fortified (September 1997 to March 1998) and designated those subjects as the study group. The availability of prefortification (base-line) data from the fifth examination and postfortification (follow-up) data from the sixth examination allowed us to assess any change in folate status that occurred with fortification in the study group. Members of the cohort whose sixth examination occurred before fortification began (January 1995 to September 1996) constituted the control group, and we used data from the fifth and sixth examinations to estimate time-related changes in folate status unrelated to fortification over a three-year period. We further divided the study and control groups into those who used vitamin supplements containing folic acid and those who did not.

Measurements

As part of the fifth and sixth examinations, blood samples were obtained after the participants had fasted (for >10 hours) to determine the concentrations of homocysteine, folate, vitamin B_{12}, and pyridoxal 5′-phosphate (the active, circulating form of vitamin B_6). Analyses of the samples from the fifth examination are complete.

The total homocysteine concentration in plasma was determined by high-performance liquid chromatography with fluorometric detection[17]; plasma folate was measured by a microbial (*Lactobacillus casei*) assay in a 96-well plate[18,19]; plasma pyridoxal 5′-phosphate was measured by the tyrosine decarboxylase apoenzyme method[20]; and plasma vitamin B_{12} was measured by a radioimmunoassay (Quantaphase II, Bio-Rad, Hercules, Calif.). Coefficients of variation for these assays were 8 percent for homocysteine, 13 percent for folate, 16 percent for pyridoxal 5′-phosphate, and 7 percent for vitamin B_{12}.

The usual dietary intake of folate was assessed with a food-frequency questionnaire.[21] This questionnaire also identified nutrient intake from dietary supplements and from fortified, ready-to-eat breakfast cereals. We included folic acid from fortified cereals with unfortified dietary sources of folate in these analyses, because we wanted to examine the added contribution from the new sources of fortification. The nutrient data base that was used for the questionnaire had not yet been modified to account for the folic acid that had recently been added to foods as part of the fortification program.

Statistical Analysis

We separated the data on subjects who reported use of supplements containing folic acid from the data on those who did not. For this reason it was necessary to exclude 242 subjects who started taking supplements containing folic acid between the fifth and sixth examinations and 95 who stopped taking them during this period.

Because the measurements of plasma homocysteine and folate, and folate intake, were positively skewed, we used log-transformed values. Inverse transformations were used to provide geometric means and their 95 percent confidence intervals. A plasma folate concentration of less than 3 ng per milliliter (7 nmol per liter) was defined as low.[22] Because there is no standard definition of a high total homocysteine concentration, we defined it for these analyses as a value of more than 13 μmol per liter, which was the 85th percentile for the cohort at the fifth examination cycle.

We determined the age- and sex-adjusted geometric means and prevalences and their 95 percent confidence intervals for the data from the fifth and sixth examinations. Because the fifth examination was completed before the implementation of fortification, measurements from this examination provided base-line values for both the study and control groups. This allowed us to examine the comparability of the groups before the study group was exposed to fortification. We used follow-up data from the sixth examination to examine the differences between the study group and the control group after the former was exposed to fortification. We used combined data from the fifth and sixth examinations to calculate the changes in folate status in the study group after exposure to fortification and in the control group over a follow-up period of similar length. We compared the base-line and follow-up values between the two groups using the SAS PROC GLM program.[23] We also used this program to test for changes between the fifth and sixth examinations within the two groups.

RESULTS

Table 1 shows the homocysteine and folate concentrations at the base-line (fifth) and follow-up (sixth) examinations for the study and control groups. The plasma folate and homocysteine concentrations at base line were not substantially different between the groups. Pyridoxal 5′-phosphate concentrations were significantly lower among subjects in the study group who did not use B vitamin supplements than among those in the control group who did not use supplements; vitamin B_{12} concentrations did not differ significantly between the groups (data not shown).

TABLE 1. PLASMA FOLATE AND HOMOCYSTEINE CONCENTRATIONS BEFORE AND AFTER FOLIC ACID FORTIFICATION IN THE FRAMINGHAM OFFSPRING STUDY COHORT, ACCORDING TO THE USE OF B VITAMIN SUPPLEMENTS.*

CHARACTERISTIC	NO B VITAMIN SUPPLEMENTS		B VITAMIN SUPPLEMENTS	
	STUDY GROUP (N=248)	CONTROL GROUP (N=553)	STUDY GROUP (N=102)	CONTROL GROUP (N=203)
Male sex — %	55	53	45	44
Age at fifth examination — yr				
Mean	57	55	56	55
Range	32–80	32–77	38–79	32–79
Plasma folate — ng/ml (95% CI)†				
Base line	4.6 (4.3–4.9)	4.6 (4.4–4.8)	11.7 (10.4–13.1)	11.4 (10.5–12.4)
Follow-up	10.0 (9.3–10.7)‡§	4.8 (4.6–5.1)	18.9 (17.0–20.9)‡§	14.1 (13.1–15.2)§
Plasma folate <3 ng/ml — % (95% CI)				
Base line	22.0 (17.3–26.7)	25.3 (22.1–28.4)	3.9 (0.0–11.2)	2.6 (0.0–7.7)
Follow-up	1.7 (0.0–5.4)‡§	20.7 (18.3–23.2)	0.0 (0.0–5.9)	0.9 (0.0–5.0)
Fasting total homocysteine — μmol/liter (95% CI)				
Base line	10.1 (9.8–10.5)	10.0 (9.8–10.2)	7.9 (7.5–8.4)	7.9 (7.6–8.3)
Follow-up	9.4 (9.1–9.7)‡§	10.2 (10.0–10.5)	8.5 (8.0–9.0)¶	8.0 (7.7–8.3)
Fasting total homocysteine >13 μmol/liter — % (95% CI)				
Base line	18.7 (14.5–22.9)	17.6 (14.8–20.4)	4.2 (0.0–10.7)	3.8 (0.0–8.4)
Follow-up	9.8 (5.6–14.0)‡§	21.0 (18.2–23.8)	7.8 (1.2–14.3)	4.2 (0.0–8.9)
Folate intake — μg/day (95% CI)‖				
Base line	266 (253–280)	275 (266–285)	650 (600–704)	651 (616–689)
Follow-up	271 (258–285)	291 (281–301)§	686 (634–743)	675 (638–714)

*The study group was examined before exposure to foods fortified with folic acid (base line) and approximately three years later, after exposure to fortification (follow-up). The control group was examined before fortification on two occasions separated by approximately three years. Base line refers to the fifth examination of the Framingham Offspring cohort (1991–1994), and follow-up to the sixth examination of the cohort (1995–1998). Folate and homocysteine values were adjusted for age and sex. CI denotes confidence interval.

†To convert values for folate to nanomoles per liter, multiply by 2.266.

‡$P<0.001$ for the comparison with the control group.

§$P<0.001$ for the comparison with the base-line value.

¶$P<0.006$ for the comparison with the base-line value.

‖The folate intake does not include the folic acid added from fortification of grain products (other than previously fortified ready-to-eat breakfast cereals).

Among the subjects in the study group who did not use B vitamin supplements, plasma folate concentrations increased by 117 percent after the introduction of folic acid fortification ($P<0.001$), the prevalence of low folate concentrations decreased by 92 percent ($P<0.001$), fasting total homocysteine concentrations decreased by 7 percent ($P<0.001$), and the prevalence of high homocysteine concentrations decreased by 48 percent ($P<0.001$) from the base-line to the follow-up examination. Among the subjects in the control group who did not take B vitamin supplements, the only significant change was an increase in reported dietary folate intake ($P<0.001$).

Among subjects in the study and control groups who used B vitamin supplements, we found a significant increase in plasma folate concentrations from the base-line examination to the follow-up examination. Plasma folate concentrations increased by 62 percent in the study group ($P<0.001$) and by 24 percent in the control group ($P<0.001$). There was also an 8 percent increase in homocysteine concentrations in the study group ($P<0.006$).

At the follow-up examination, mean homocysteine concentrations were 10 percent lower among those in the study group who used supplements than among those who did not use supplements ($P<0.001$), but the prevalence of high homocysteine concentrations was not significantly different between these two subgroups ($P=0.62$). The difference in mean homocysteine concentrations appears to be largely the result of differences in vitamin B_{12} and pyridoxal 5′-phosphate status between those who used B vitamin supplements and those who did not. Mean plasma vitamin B_{12} concentrations were 351 pg per milliliter (259 pmol per liter) in those who did not use supplements and 475 pg per milliliter (350 pmol per liter) in those who did ($P<0.001$). Similarly, the pyridoxal 5′-phosphate concentrations were 53 nmol per

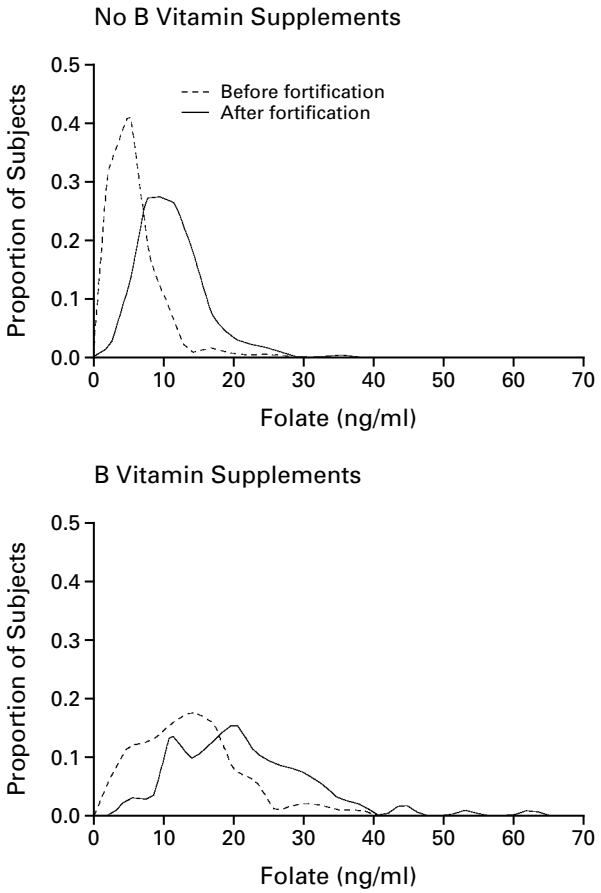

Figure 1. Plasma Folate Concentrations in the Study Group before and after Folic Acid Fortification, According to the Use of B Vitamin Supplements.

A total of 102 subjects used B vitamin supplements, and 248 did not. To convert values for folate to nanomoles per liter, multiply by 2.266.

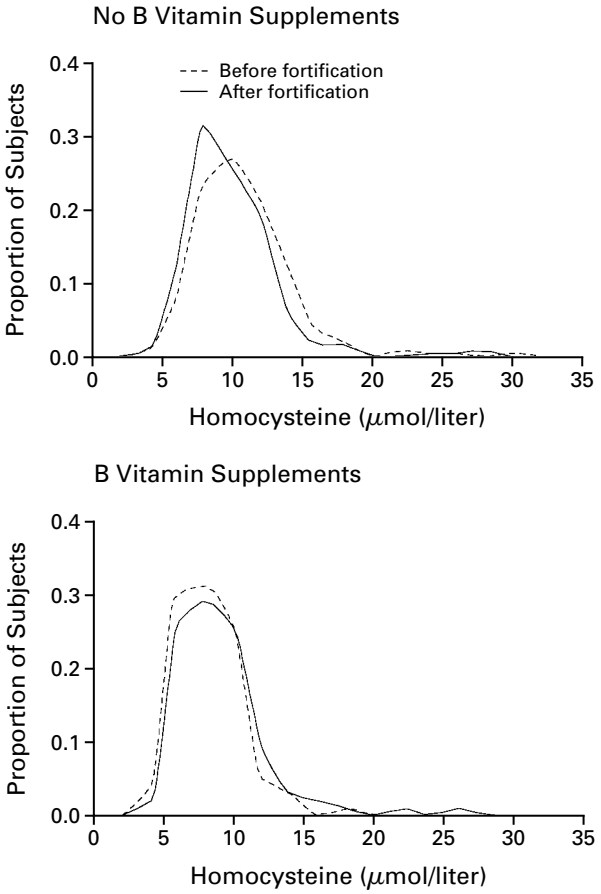

Figure 2. Plasma Total Homocysteine Concentrations in the Study Group before and after Folic Acid Fortification, According to the Use of B Vitamin Supplements.

A total of 102 subjects used B vitamin supplements, and 248 did not.

liter in those who did not use supplements and 120 nmol per liter in those who did ($P<0.001$). After we adjusted for vitamin B_{12} and pyridoxal 5′-phosphate concentrations, the difference in homocysteine concentrations between those in the study group who used supplements and those who did not was reduced to 6 percent and was no longer statistically significant ($P=0.10$). In the study group, the prevalence of high homocysteine concentrations was essentially the same for those who used B vitamin supplements and those who did not after adjustment for vitamin B_{12} and pyridoxal 5′-phosphate concentrations ($P=0.83$).

Figures 1 and 2 show the plasma folate and homocysteine concentrations, respectively, at the baseline and follow-up examinations in the study group, according to the use of B vitamin supplements. Figure 1 shows the upward shift in the distribution of plasma folate concentrations from the base-line (prefortification) examination to the follow-up (postfortification) examination for both those who used supplements and those who did not. Figure 2 illustrates the decrease in the area of the upper tail of homocysteine distribution after fortification and the increase in the height of the distribution of normal homocysteine concentrations (<10 μmol per liter) among those who did not use supplements. There was a slight upward shift in homocysteine concentrations between examinations among those who used supplements.

DISCUSSION

Our findings suggest that folic acid fortification has had a substantial effect on plasma folate and homocysteine concentrations in a population-based sample of middle-aged and older adults. Low folate concentrations (<3 ng per milliliter) were largely eliminated in this population after folic acid fortification was implemented, and the prevalence of high homocysteine concentrations (>13 μmol per liter) was re-

duced by approximately 50 percent among those who did not take supplements. The differences in values between those who were exposed to fortification and those who were not exposed appear to be specific for folate. Furthermore, these differences cannot be attributed to changes in folate intake from sources other than folic acid added to the diet as part of fortification.

Although the apparent effect of fortification on plasma folate and homocysteine concentrations was striking, the concentrations of folate were significantly higher, and concentrations of homocysteine significantly lower, among subjects who used vitamin supplements that contained folic acid. The consequences of these differences are not entirely clear. Although the mean folate concentrations among subjects who were exposed to folic acid fortification were higher among those who used supplements than among those who did not, the prevalence of low folate concentrations was very low in both groups and was not significantly different between groups. Among the subjects who were exposed to foods fortified with folic acid, mean homocysteine concentrations were lower in those who used supplements than in those who did not, but this difference did not clearly translate into a difference in the prevalence of high homocysteine concentrations. Approximately 10 percent of those who were not taking supplements had high homocysteine concentrations in the postfortification period, but this prevalence was not significantly different from the approximately 8 percent prevalence of high homocysteine concentrations in those who used supplements.

Moreover, the differences in mean homocysteine concentrations between those who used supplements and those who did not cannot be attributed readily to folate status. There were substantial differences between these two groups in concentrations of vitamin B_{12} and pyridoxal 5′-phosphate (the active, circulating form of vitamin B_6), which are the other important vitamins that determine the concentration of homocysteine. Such a difference can be expected, because all the supplements containing folic acid were either multivitamins or B-complex vitamins that contained vitamins B_{12} and B_6. Thus, any unadjusted comparison of homocysteine concentrations as a measure of folate status between those who used supplements and those who did not is confounded. When we controlled for vitamin B_{12} and pyridoxal 5′-phosphate concentrations in the analyses, the difference in homocysteine concentrations between those who used supplements and those who did not was reduced substantially and was no longer statistically significant. These data suggest that the higher mean homocysteine concentrations in those who did not use supplements and who were seen during the postfortification period were probably not a consequence of inadequate folate intake. These data provide little evidence that the addition of 400 μg of folic acid per day from supplements to the amount provided by fortification and diet further reduced homocysteine concentrations, but our ability to detect small differences resulting from the additional folic acid is limited by the small number of persons who used supplements and who were exposed to fortification.

It was predicted that folic acid fortification at a level of 140 μg per 100 g would provide an additional 70 to 120 μg of folic acid per day for middle-aged and older adults.[12] A recent study examined the effect of three levels of folic acid added to breakfast cereal on plasma total homocysteine and folate concentrations[24] and concluded that an additional 100 μg of folic acid per day was not sufficient to minimize total homocysteine concentrations. However, features of that study may limit the applicability of the observation to the general population with long-term exposure to folic acid fortification. The length of treatment was only five weeks, which was probably insufficient to approach a new steady-state concentration at a dose of 100 μg per day,[25] and the study was performed in patients with coronary artery disease, who may require a higher folate intake to minimize total homocysteine concentrations.[26] The issue of the length of exposure to foods fortified with folic acid was highlighted in a report by Schorah and colleagues.[27] They found that folate concentrations in serum and red cells continued to increase and that homocysteine concentrations continued to decrease 8 weeks after the addition of 200 μg of folic acid per day to breakfast cereal, and possibly up to 24 weeks afterward. We must also consider the possibility that enriched grain products are being fortified at levels above the minimum required by the FDA (140 μg per 100 g of cereal or grain product). However, preliminary data on the folic acid content of enriched grain products suggest that this is probably not the case.[28] Tests of common national brands of enriched flour, pasta, and rice that are available in the Framingham area revealed that folic acid concentrations ranged from 125 to 136 μg per 100 g in flour, 180 to 205 μg per 100 g in pasta, and 66 to 176 μg per 100 g in rice.

Folic acid fortification was undertaken to reduce the risk of neural-tube defects,[1,2] but it may also have a beneficial effect on vascular disease because of the relation between inadequate folate intake and higher circulating homocysteine concentrations.[29,30] Elevated fasting total homocysteine concentrations are clearly amenable to treatment with folic acid,[31-34] and elevated concentrations of circulating total homocysteine,[30,35-39] as well as lower folate intake and status,[40-42] are associated with an increased risk of occlusive vascular disease. If a high concentration of homocysteine ultimately proves to be a risk factor for vascular disease, our data indicate that folic acid fortification

would have a measurable effect on the rates of cerebrovascular and coronary heart disease in the United States. Only a small proportion of our population was made up of women younger than 40, so we were not able to assess directly the effect of fortification on women of reproductive age. However, we have no reason to believe that the effect of fortification on folate status in women of reproductive age differs from the effect in older adults.

Supported in part by an agreement (58-1950-9-001) with the Department of Agriculture and by a contract (N01-HC-38038) with the National Heart, Lung, and Blood Institute. Any opinions, findings, conclusions, or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of the Department of Agriculture.

REFERENCES

1. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Fed Regist 1996;61(44):8781-97.
2. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Morb Mortal Wkly Rep 1992;41(RR-14):1-7.
3. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 1991; 338:131-7.
4. Czeizel AE. Prevention of congenital abnormalities by periconceptional multivitamin supplementation. BMJ 1993;306:1645-8.
5. Laurence KM, James N, Miller MH, Tennant GB, Campbell H. Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. BMJ 1981;282:1509-11.
6. Vergel RG, Sanchez LR, Heredero BL, Rodriguez PL, Martinez AJ. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. Prenat Diagn 1990;10:149-52.
7. Smithells RW, Nevin NC, Seller MJ, et al. Further experience of vitamin supplementation for prevention of neural tube defect recurrences. Lancet 1983;1:1027-31.
8. Werler MM, Shapiro S, Mitchell AA. Periconceptional folic acid exposure and risk of occurrent neural tube defects. JAMA 1993;269:1257-61.
9. Mulinare J, Cordero JF, Erickson JD, Berry RJ. Periconceptional use of multivitamins and the occurrence of neural tube defects. JAMA 1988;260: 3141-5.
10. Milunsky A, Jick H, Jick SS, et al. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA 1989;262:2847-52.
11. Bower C, Stanley FJ. Dietary folate as a risk factor for neural-tube defects: evidence from a case-control study in Western Australia. Med J Aust 1989;150:613-9.
12. Food standards: amendment of the standards of identity for enriched grain products to require addition of folic acid. Fed Regist 1993;58(197): 53305-12.
13. Oakley GP Jr. Eat right *and* take a multivitamin. N Engl J Med 1998; 338:1060-1.
14. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693-8.
15. Dawber TR, Moore FE, Mann GV. Coronary heart disease in the Framingham study. Am J Public Health 1957;47:4-24.
16. Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study: design and preliminary data. Prev Med 1975;4:518-25.
17. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422:43-52.
18. Horne DW, Patterson D. *Lactobacillus casei* microbiological assay of folic acid derivatives in 96-well microtiter plates. Clin Chem 1988;34:2357-9.
19. Tamura T, Freeberg LE, Cornwell PE. Inhibition by EDTA of growth of *Lactobacillus casei* in the folate microbiological assay and its reversal by added manganese or iron. Clin Chem 1990;36:1993.
20. Shin YS, Rasshofer R, Friedrich B, Endres W. Pyridoxal-5′-phosphate determination by a sensitive micromethod in human blood, urine and tissues: its relation to cystathioninuria in neuroblastoma and biliary atresia. Clin Chim Acta 1983;127:77-85.
21. Rimm ED, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114-26.
22. Herbert V, Das KC. Folic acid and vitamin B_{12}. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Vol. 1. Philadelphia: Lea & Febiger, 1994:402-25.
23. SAS user's guide, version 6. Cary, N.C.: SAS Institute, 1989.
24. Malinow MR, Duell PB, Hess DL, et al. Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. N Engl J Med 1998;338:1009-15.
25. Gregory JF, Bailey LB, Toth JP, Williamson J. Kinetic model of folate metabolism in nonpregnant women chronically given [$^2H_2$]folic acid with three levels of total folate intake. FASEB J 1998;12:A511. abstract.
26. Hopkins PN, Wu LL, Wu J, et al. Higher plasma homocyst(e)ine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease. Arterioscler Thromb Vasc Biol 1995;15:1314-20.
27. Schorah CJ, Devitt H, Lucock M, Dowell AC. The responsiveness of plasma homocysteine to small increases in dietary folic acid: a primary care study. Eur J Clin Nutr 1988;52:407-11.
28. Afman L, Bagley P, Selhub J. Determination of folic acid in fortified cereal products using the affinity/HPLC method. FASEB J 1999;13:A891. abstract.
29. Tucker KL, Mahnken B, Wilson PWF, Jacques P, Selhub J. Folic acid fortification of the food supply: potential benefits and risks for the elderly population. JAMA 1996;276:1879-85. [Erratum, JAMA 1997;277:714.]
30. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA 1995;274:1049-57.
31. Brattström LE, Israelsson B, Jeppsson J-O, Hultberg BL. Folic acid — an innocuous means to reduce plasma homocysteine. Scand J Clin Lab Invest 1988;48:215-21.
32. Wilcken DEL, Dudman NPB, Tyrrell PA, Robertson MR. Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: possible implications for prevention of vascular disease. Metabolism 1988;37: 697-701.
33. Landgren F, Israelsson B, Lindgren A, Hultberg B, Andersson A, Brattström L. Plasma homocysteine in acute myocardial infarction: homocysteine-lowering effect of folic acid. J Intern Med 1995;237:381-8.
34. Ubbink JB, Vermaak WJH, van der Merwe, Becker PJ, Delport R, Potgieter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. J Nutr 1994;124:1927-33.
35. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 1992;268:877-81.
36. Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. Int J Epidemiol 1995;24:704-9.
37. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995;346:1395-8.
38. Graham IM, Daly LE, Refsum HM, et al. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. JAMA 1997;277:1775-81.
39. Nygård O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230-6.
40. Rimm EB, Willett WC, Hu FB, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA 1998;279:359-64.
41. Verhoef P, Stampfer MJ, Buring JE, et al. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. Am J Epidemiol 1996;143:845-59.
42. Morrison HI, Schaubel D, Desmeules M, Wigle DT. Serum folate and risk of fatal coronary heart disease. JAMA 1996;275:1893-6.