Dietary Supplement Use in Women:
Current Status and Future Directions

Folic Acid Supplements and Fortification Affect the Risk for Neural Tube Defects, Vascular Disease and Cancer: Evolving Science[1,2]

Lynn B. Bailey,[3] Gail C. Rampersaud and Gail P. A. Kauwell

Food Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611-0370

ABSTRACT Folic acid supplements reduce the risk of neural tube defects and may be associated with reduced risk for vascular disease and cancer. Research data from both observational and controlled intervention studies provide strong support for the existing public health policies related to folic acid and neural tube defects. However, educational efforts to promote daily intake of folic acid supplements by women of reproductive age have not, in most cases, resulted in increased supplement use. In contrast, food fortification appears to be associated with a reduction in neural tube defects in the United States and Canada but is not practiced universally. The potential for folic acid supplements to reduce the incidence and severity of vascular disease and cancer is the focus of major research efforts including ongoing intervention studies. J. Nutr. 133: 1961S–1968S, 2003.

KEY WORDS: • *folic acid* • *supplements* • *neural tube defects* • *vascular disease* • *cancer*

Folic acid is the fully oxidized monoglutamyl form of this water-soluble vitamin that is used commercially in supplements and in fortified foods. Metabolically, folic acid is converted to coenzyme forms required in numerous one-carbon transfer reactions involved in the synthesis, interconversion and modification of nucleotides, amino acids and other essential structural and regulatory compounds [**Fig. 1**; (1)].

Folic acid supplements taken periconceptionally have been definitively proven to significantly reduce the risk of neural tube defects (NTD) (2–4). The conclusive scientific evidence related to folic acid supplements and NTD risk reduction has been translated into public health policy (5,6). The link between folic acid supplements and vascular disease risk is associated with the proven effectiveness of folic acid to significantly reduce plasma homocysteine concentration, a known vascular disease risk factor (7,8). Risk for certain cancers may be inversely associated with long-term use of multivitamins containing folic acid (9) and with low folate intake especially when coupled with moderate alcohol intake (10,11). The objective of this article is to present an overview of the evolving science related to folic acid supplements and risk for NTD, vascular disease and cancer.

Neural tube defects

The evidence that supplements containing folic acid that are taken periconceptionally dramatically reduce the risk of NTD is so strong that a series of public health policies have been implemented worldwide (5,6,12–15). The evolution of science that preceded these policies was recently summarized (16) and represents a model to demonstrate how a body of scientific data supports such policies. The research evidence on which these policies are based includes that from both observational studies and intervention trials, the most definitive of which was from an international multicenter-randomized trial in which the dramatic effect (72% risk reduction) of periconceptional folic acid supplements on NTD recurrence was demonstrated (4).

After the MRC study (4), a folic acid intervention trial in Hungary provided definitive evidence that supplements containing folic acid significantly reduce the risk of NTD occurrence (3). Data from the intervention trials strongly supported those from earlier observational studies (17–20), which led scientists and public health policy makers to accept the conclusion that supplemental periconceptional folic acid use significantly reduces NTD risk (16). These decisive data were translated into a number of public health policies including the 1992 U.S. Public Health Service (5) and the 1998 Institute of Medicine recommendation (6) that all women of reproductive age (5) or capable of becoming pregnant (6)

[1] From the National Institutes of Health (NIH) conference "Dietary Supplement Use in Women: Current Status and Future Directions" held on January 28–29, 2002, in Bethesda, MD. The conference was sponsored by the National Institute of Child Health and Human Development and the Office of Dietary Supplements, NIH, U.S. Department of Health and Human Services (DHHS) and was cosponsored by the Centers for Disease Control and Prevention, Food and Drug Administration Office of Women's Health, NIH Office of Research on Women's Health, National Institute of Diabetes and Digestive and Kidney Diseases Division of Nutrition Research Coordination, DHHS; National Center for Complementary Medicine, U.S. Department of Agriculture Agricultural Research Service; International Life Sciences Institute North America; March of Dimes; and Whitehall Robbins Healthcare. Conference proceedings were published in a supplement to *The Journal of Nutrition*. Guest editors for this workshop were Mary Frances Picciano, Office of Dietary Supplements, NIH, DHHS; Daniel J. Raiten, Office of Prevention Research and International Programs, National Institute of Child Health and Human Development, NIH, DHHS; and Paul M. Coates, Office of Dietary Supplements, NIH, DHHS.

[2] Florida Agricultural Experiment Station Journal Series Number R-09432.

[3] To whom correspondence should be addressed.
E-mail: LBBailey@mail.ifas.ufl.edu.

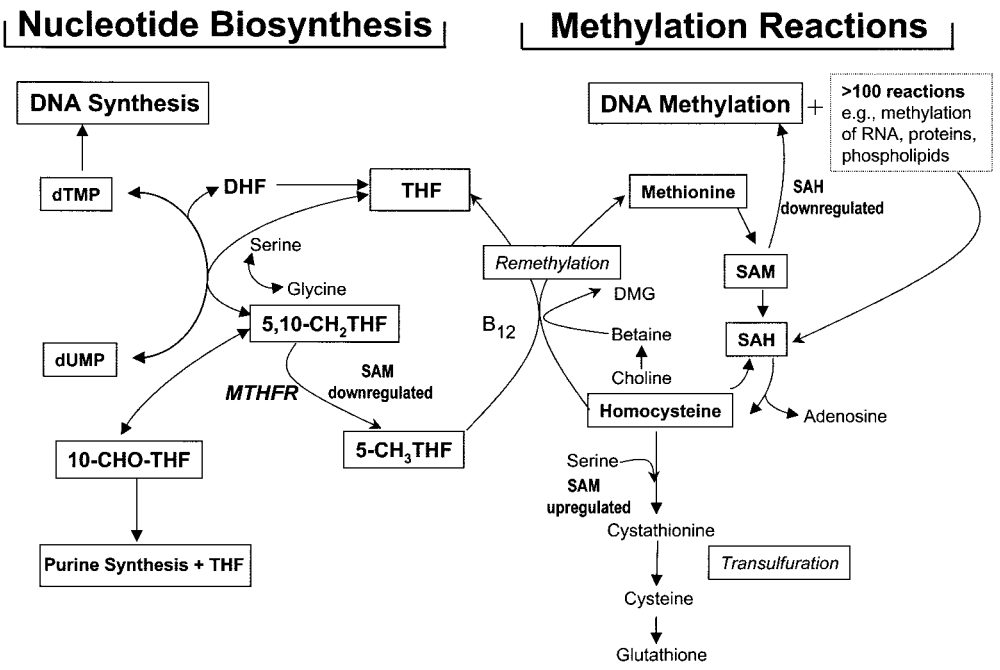

FIGURE 1 Folate metabolic cycle. Abbreviations: DHF = dihydrofolate; dTMP = deoxythymidylate monophosphate; DMG = dimethylglycine; dUMP = deoxyuridylate monophosphate; MTHFR = methylenetetrahydrofolate reductase enzyme; 5,10-CH_2THF = 5,10-methylenetetrahydrofolate; 5-CH_3THF = 5-methyltetrahydrofolate; 10-CHO-THF = 10-formyltetrahydrofolate; SAM = *S*-adenosylmethionine; SAH = *S*-adenosylhomocysteine; THF = tetrahydrofolate. Reprinted from Bailey et al. (1) with permission.

consume 400 μg/d folic acid from supplements (5) or fortified foods (6). The Institute of Medicine recommendation additionally states that intake of folic acid from supplements or fortified foods should be coupled with consumption of food folate from a varied diet (6).

The U.S. Public Health Service folic acid–NTD public health policy was followed by a Food and Drug Administration regulation requiring food manufacturers to fortify all enriched cereal grain products with folic acid by January 1, 1998 (13). Mandatory fortification has also been implemented in a limited number of countries outside of the United States, including Canada (14) and Chile (21). The selection of 400 μg/d as the recommended supplemental dose was based on the fact that this is the quantity included in most multivitamin supplements associated with NTD risk reduction in large observational studies (19,20). In contrast, the dose used in the MRC and Hungarian intervention trials was much higher (4000 and 800 μg/d, respectively). Subsequently, evidence of the effectiveness of the 400 μg/d dose was provided by a large-scale community intervention trial (n = ~250,000) in northern and southern China in which NTD rates were compared in women who either did or did not take a daily 400 μg folic acid supplement periconceptionally (2). The reduction in NTD risk associated with folic acid intake (**Fig. 2**) was ~80% in northern China where the prevalence of NTD was high. A lower but also significant reduction (41%) was observed in the southern region where the prestudy NTD prevalence was lower and similar to that observed in the United States (1/1000 births).

Conclusions regarding the effectiveness of public health policies related to folic acid fortification and periconceptional folic acid vitamin use include a number of reports documenting significant improvement in folate status in the United States and Canada and several reports suggesting a significant reduction in NTD prevalence in the United States, Canada and Western Australia. Folic acid fortification has clearly improved folate status both in the United States (22–25) and in Canada (26). A comparison of prefortification data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) with the 1999 NHANES (postfortification) indicated that the mean serum folate concentration for women aged 15–44 y who did not use supplements increased from 10.7 nmol/L to 28.6 nmol/L shortly after the initiation of fortification in the United States, representing an almost threefold increase (**Fig. 3**) (22). The increase appears to be associated with consumption of folic acid in fortified foods (enriched grain products and fortified ready-to-eat breakfast cereals) because the data for supplement users were evaluated separately (Fig. 3). Similarly, a comparison of pre- and postfortification serum and red blood cell folate concentrations from the Framingham Offspring Cohort indicated increases in serum folate concentration in men and women from 10.4 to 22.7 nmol/L (24) and red blood cell folate concentration from 737 to 1020 nmol/L (23) in individuals not taking vitamin supplements. The effect of mandatory food fortification in Canada was evaluated in women of reproductive age (18–42

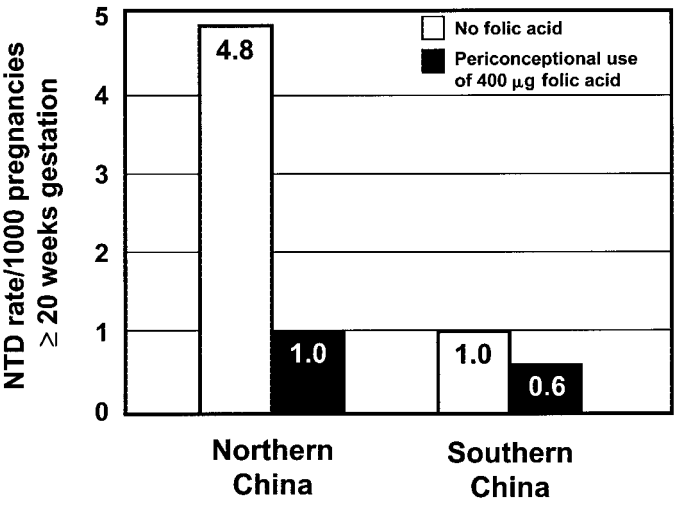

FIGURE 2 Rates of neural tube defects in northern and southern China according to periconceptional use of folic acid supplements. Data from Berry et al. (2).

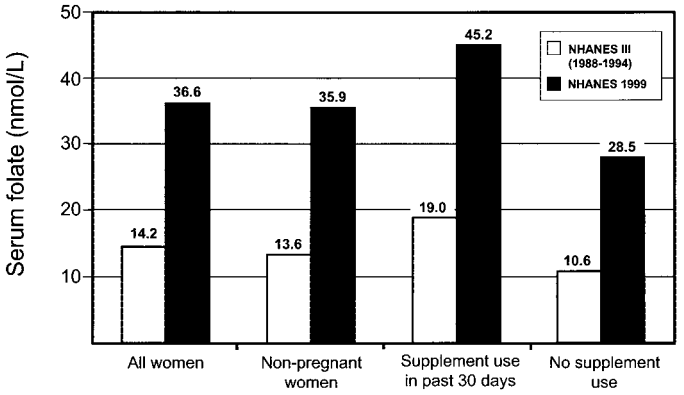

FIGURE 3 Impact of folic acid fortification on serum folate status in U.S. women of childbearing age (18–44 y). Data from the third National Health and Nutrition Examination Survey (NHANES III) represent the prefortification period and data from NHANES 1999 represent the postfortification period. Data from Centers for Disease Control and Prevention (22).

y) from January 1, 1996 to December 31, 1997, (prefortification) and January 1, 1998 to December 31, 2000 (postfortification) (26). The geometric mean red blood cell folate concentration rose from 527 nmol/L prefortification to 741 nmol/L postfortification. In Western Australia, where limited voluntary folic acid fortification was approved for specified foods in 1995 coupled with educational campaigns to increase periconceptional folic acid use, mean serum folate concentration was estimated to increase ~19% after voluntary fortification (27).

The potential effect of folic acid fortification on NTD occurrence and prevalence in the United States was estimated in two studies (28,29). Honein et al. (28) evaluated data from birth certificates from 45 states and Washington, D.C., and reported the number of infants whose birth certificates indicated they were affected by either spina bifida or anencephaly. These data suggested a 19% reduction in NTD birth prevalence when comparing births during the prefortification period (October 1995–December 1996) with births that would have been exposed periconceptionally to folic acid fortification (i.e., births occurring from October 1998 through December 1999). Specifically, the data indicated that the prevalence of spina bifida and anencephaly declined 23% and 11%, respectively, although the percent decline for anencephaly was not statistically significant. Because these data are for live births only, NTD-affected infants who were either miscarried or stillborn or affected pregnancies that were electively terminated were not considered in this analysis. Recently, Williams et al. (29) evaluated NTD prevalence during pre- and postfortification (January 1995 through December 1996, October 1998 through December 1999, respectively). Population-based NTD birth surveillance data were collected from 23 states and Puerto Rico. Nine of the 24 programs included prenatally diagnosed NTD in their ascertainment. Compared with prefortification, the prevalence of spina bifida and anencephaly decreased by 31% and 16%, respectively. When data for programs with prenatal ascertainment were compared with no prenatal ascertainment, the conclusion for spina bifida did not change appreciably in contrast to the trend for anencephaly, which became nonsignificant.

In Canada, analysis of the data from the Canadian Congenital Anomalies Surveillance System along with hospital data on therapeutic abortions indicated that the incidence of NTD decreased by 47% in Ontario from 1995 to 1999 (30). Based on a retrospective study of live births, stillbirths and terminated pregnancies as documented in perinatal and fetal anomaly databases, incidence of NTD decreased 54% in Nova Scotia after folic acid fortification (31). In Western Australia a 30% reduction in NTD was estimated from 1996 to 2000 data in the Western Australia Birth Defects Registry (32). This decrease followed health promotion campaigns encouraging periconceptional folic acid supplementation and voluntary fortification of selected food items.

In contrast to the increase in folic acid intake associated with consumption of fortified foods, there does not appear to be a comparable change in folic acid supplement use among women of childbearing age in response to the public health policy recommendations advocating daily consumption of supplements containing folic acid. A recent March of Dimes Survey showed that only 33% of women of reproductive age (18–45 y) take a daily supplement containing folic acid, representing a very modest increase from the percentage of women (28%) who reported doing so in 1995 (33). Similar data were reported in Puerto Rico, where a nationwide folic acid promotion program has been in effect for the past 4 y. Although 88% of all pregnant women (i.e., those who planned and those who did not plan their pregnancy) had knowledge of the importance of folic acid, only 32% took a supplement containing folic acid during the periconceptional period (34). A survey of Dutch pregnant women indicated that use of folic acid during the entire periconceptional period was 36% and ranged from 26% to 47% depending on level of education (35). The survey was conducted after the Dutch folic acid campaign. In Western Australia ~30% of pregnant women reported taking folic acid during the periconceptional period (36). The NTD Intervention Awareness Campaign conducted in South Carolina provides some evidence of success regarding reductions in NTD rates and increased compliance with recommendations to take folic acid supplements in targeted geographical areas. The overall NTD rates were significantly reduced and no NTD recurrences were reported in women with a previous NTD-affected pregnancy who consumed supplements containing folic acid periconceptionally (37). The drop in overall NTD rates preceded fortification and coincided with higher reported supplemental folic acid intakes.

NTD incidence appears to have fallen in the United States by ~15–30%, and to an even greater degree in Canada, since the initiation of fortification, although conclusions from these studies are not directly comparable because of methodological differences. Food fortification was originally proposed to provide a portion of the dose (400 μg/d) previously associated with NTD risk reduction. To achieve the maximal possible NTD risk reduction, estimated to be ~70% (12), it was intended that consumption of enriched cereal grain products by women of reproductive age would be coupled with increased folic acid supplement use and intake of folate-rich foods (e.g., orange juice, dark green leafy vegetables, asparagus, dried beans, peanuts, strawberries).

Even though food fortification has been associated with reduced NTD rates, this public health approach continues to be controversial because of a continuing concern that additional folic acid in the diets of population groups not originally targeted for fortification may have adverse effects (38–40). Folic acid is not associated with toxicity, and the concern relates to the potential ability of folic acid supplementation to mask the diagnosis of a vitamin B-12 deficiency, a condition that affects 10–15% of the population over age 60 (6,41). The Tolerable Upper Intake Level of 1000 μg/d of synthetic folic acid established for the new folate Dietary Reference Intakes was based solely on the risk associated with masking the

diagnosis of a vitamin B-12 deficiency (6). Currently, no European country requires mandatory folic acid fortification of flour. In 2000 the United Kingdom's Committee on Medical Aspects of Food and Nutrition Policy proposed mandatory folic acid fortification of flour at a concentration of 240 mg/100 g flour, almost twice the U.S. fortification level (42). However, the United Kingdom's Food Standards Agency Board recently decided against mandatory folic acid fortification, in part because of the potential for masking the diagnosis of a vitamin B-12 deficiency (40,43). The Dutch Health Council recommended against mandatory fortification for similar reasons (44).

In countries without mandatory food fortification, women will need to depend solely on periconceptional supplementation of folic acid for NTD risk reduction. The effectiveness of this strategy to significantly reduce NTD risk is uncertain because data indicate that folic acid awareness has not translated into behavior change (3). Therefore, the need to fortify foods in countries not currently implementing mandatory fortification appears to be the most rational and feasible approach (40), especially because fortification has now been associated with documented decreases in NTD incidence in the United States and Canada (28,29,31).

Vascular disease

Numerous epidemiologic studies support an inverse association between dietary folate intake and vascular disease risk. The metabolic basis for the observed inverse association appears to be related to the fact that folate is a coenzyme in the regulation of normal plasma homocysteine concentrations through a key remethylation reaction (Fig. 1). Hyperhomocysteinemia, characterized as an elevation in blood homocysteine concentration, is considered a significant risk factor for atherosclerotic vascular disease in the coronary, cerebral and peripheral vessels and for arterial and venous thromboembolism (7,45). Numerous cross-sectional, case-control and prospective studies associate elevated blood homocysteine concentrations with increased risk for coronary heart (**Fig. 4**) and cerebrovascular disease (46). A meta-analysis of observational studies examining the relationship between blood homocysteine and vascular disease risk suggested that a sustained lowering of homocysteine concentration by 1 μmol/L is associated with a ~10% reduction in vascular disease risk throughout a range of homocysteine concentrations (10–15 μmol/L) (7).

Folic acid and to a lesser extent vitamins B-6 and B-12 have a homocysteine-lowering effect, the size of which depends on pretreatment blood homocysteine and folate concentrations. The Homocysteine Lowering Trialists' Collaboration (47) estimated the size of the reduction in blood homocysteine achieved with different doses of folic acid by a meta-analysis of randomized trials. Individuals with higher pretreatment homocysteine concentrations or low blood folate concentrations benefited the most from folic acid supplementation. However, after standardizing pretreatment blood concentrations of homocysteine and folate in the meta-analysis (to 12 μmol/L and 12 nmol/L, respectively), folic acid doses of <1, 1–3 and >3 mg/d were each associated with comparable reductions in blood homocysteine of ~25% (**Fig. 5**). There appears to be a plateauing effect on blood homocysteine concentrations with folic acid doses of ~400–500 μg/d (7,47,48). In individuals with lower homocysteine concentrations, similar to those observed after folic acid fortification (~8–10 μmol/L) (24), supplemental folic acid also was shown to result in a significant reduction in homocysteine concentration (49). However, the size of the reduction in individuals with low–normal (~8–10 μmol/L) homocysteine concentration would be expected to be considerably less than that in individuals with higher baseline concentrations such as those currently observed in European countries (47,50–52).

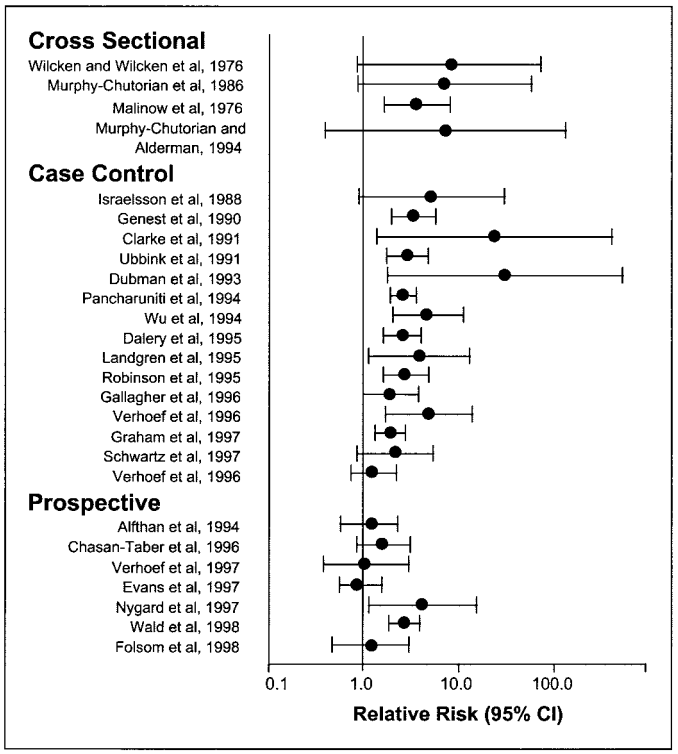

FIGURE 4 Summary of relative risks and 95% confidence intervals (CI) from studies evaluating the association between elevated blood homocysteine concentrations and risk of coronary heart disease. Complete citations for references cited in this figure are included in (46). Reproduced from Christen et al. (46) with permission.

A major unresolved question is whether supplemental folic acid will reduce the overall incidence, morbidity and mortality of vascular disease. Data from controlled intervention trials like those supporting the role of folic acid in NTD risk reduction are not available. Data support a role for folic acid in improving subclinical markers of vascular disease risk, such as endothelial dysfunction (53–56) and abnormal exercise electrocardiography tests, when folic acid is coupled with vitamin B-6 (57). In a randomized-controlled intervention study, a significant reduction in target lesion revascularization and other adverse clinical events after percutaneous coronary intervention was observed in response to a combined supplement containing folic acid, vitamin B-6 and vitamin B-12 (58). However, it is still unclear whether improvement in these clinical indicators of vascular disease will translate into reduced incidence of vascular disease.

A series of ongoing large-scale randomized-controlled intervention trials are underway in the United States, Canada, Europe and Australia to assess the effect of folic acid and other B vitamin supplementation on vascular disease incidence (8). It is uncertain whether the results of these trials will be definitive regarding the effect of folic acid on disease risk because of a number of confounding factors. For example, because folic acid fortification has been associated with a significant reduction in plasma homocysteine concentration, subjects included in randomized studies in the United States and Canada may have lower baseline homocysteine concentrations than originally anticipated when the studies were

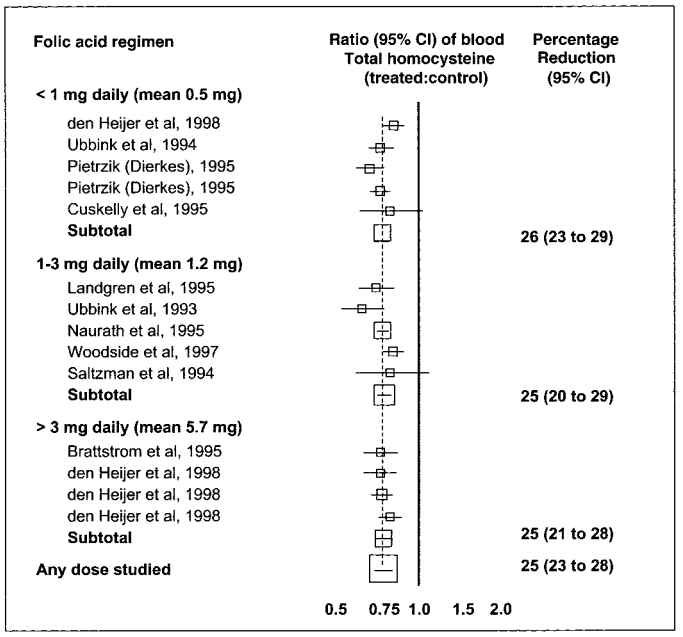

FIGURE 5 Blood homocysteine concentration response to various folic acid supplementation regimens. Squares represent blood homocysteine ratio between folic acid treated and control subjects. Subjects' pretreatment serum and blood homocysteine concentrations were standardized to 12 nmol/L and 12 μmol/L, respectively. Complete citations for references cited in this figure are included in (47). Reprinted from Homocysteine Lowering Trialists' Collaboration (47) with permission.

designed. As hypothesized by Bostom et al. (59), these trials may not achieve the same reduction in homocysteine that would occur in a population not exposed to fortification and therefore may not have the statistical power necessary to detect a significant effect on disease outcome.

A second factor that will interfere with the interpretation of the effect of folic acid alone in these trials is the fact that, with several exceptions, folic acid is given in combination with other nutrients that may significantly affect homocysteine concentration and thus vascular disease risk. Finally, the etiology of vascular disease is multifactorial, which may further confound results of these secondary intervention trials. Therefore, the degree to which public health policy regarding folic acid and vascular disease will be shaped by the results of ongoing intervention trials remains to be seen.

Cancer

A relationship between folate intake or status and several types of cancers, including colorectal, breast, cervical, pancreatic, brain and lung cancers, has been observed in several population-based studies (60). This relationship is most clearly defined for colorectal cancer and its precancerous condition, colorectal adenomas. Small-scale intervention studies indicate that folic acid supplementation may improve biomarkers of colorectal dysplasia. In placebo-controlled studies using patients with a history of colonic dysplasia or neoplasia, folic acid supplements improved DNA methylation status in colonic or rectal mucosa (61,62) and reduced colonic mucosal cell proliferation (63). Data from case-control and cohort studies support an inverse association between folate intake or status and risk for colorectal adenomas or cancer [**Fig. 6**; (64)].

Lower risks for disease are commonly observed in individuals with higher folate intake or status, and individuals in higher intake groups typically are users of multivitamins or supplements. Therefore, folic acid from supplement sources can be an important contributor to overall folate intake in the diet and may play a role in contributing to cancer risk reduction. Giovannucci et al. (65) evaluated the risk of colorectal adenomas in the Nurses' Health Study (15,984 women) and Health Professionals Follow-Up Study (9490 men) cohorts. A 30–40% decreased risk for colorectal adenomas was found with median energy-adjusted total folate intakes >700 μg/d compared with the lowest folate intake group (166 and 241 μg/d for women and men, respectively). Although the percentage of supplement users in the highest quintile of intake was not stated, it is presumed to be relatively high because of the amount of intake. In this study, folate intake from foods only was not significantly associated with decreased risk. When the association between folate intake and the more advanced stage of colorectal cancer was examined in the Nurses's Health Study, a 30% lower risk was associated with consumption of ≥400 μg/d of total folate compared with

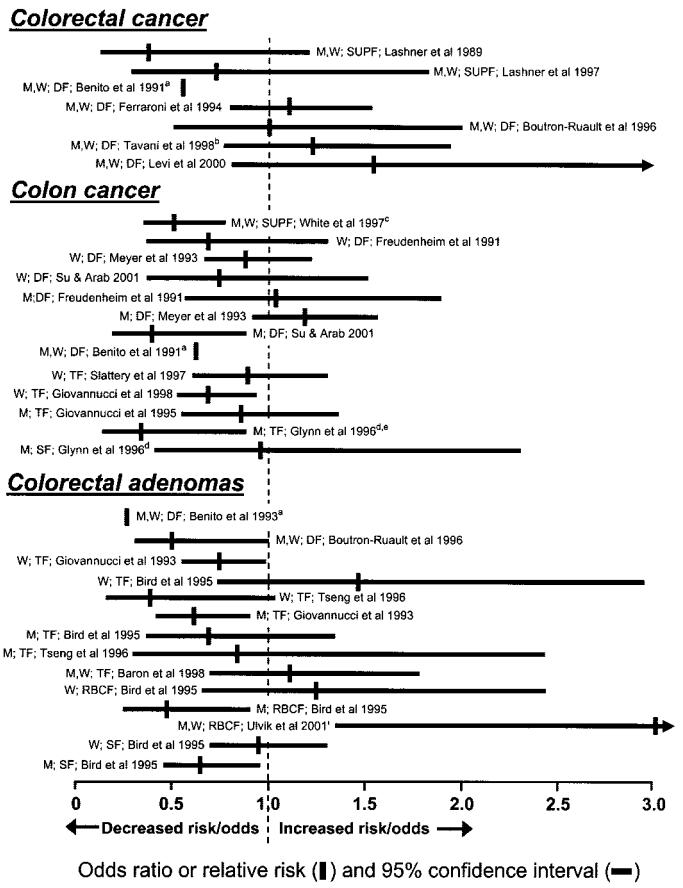

FIGURE 6 Relative risk or odds ratio and 95% confidence intervals for epidemiologic studies evaluating an association between colorectal adenomas or neoplasia and folate intake or status. Unless otherwise noted, risks represent highest versus lowest folate intake or status group. Descriptive labels include gender group, variable of interest, and author/y published. Complete citations for references cited in this figure are included in (64). Abbreviations: DF = dietary folate; M = men; RBCF = red blood cell folate; SF = serum folate; SUPF = supplemental folate; TF = total folate; W = women. [a] No 95% confidence interval reported. [b] Ex-drinkers versus never-drinkers. [c] Folic acid from multivitamins only. [d] Smokers. [e] Odds ratio is for the 3rd quartile of folate intake. [f] Odds ratio is for the lowest versus highest tertile of RBCF. Reprinted from Rampersaud et al. (64) with permission.

women in the lowest folate intake groups (≤200 μg/d) (9). Over 86% of women in the highest folate intake category used multivitamins. When duration of multivitamin use was evaluated, a dramatic 75% lower risk for colon cancer was observed in women who had used multivitamins containing folic acid for at least 15 y compared with nonusers (**Fig. 7**). There was no significant benefit associated with women taking multivitamins for a shorter period of time.

Other data have confirmed risk reductions in colon (66) and cervical (67) cancer risk with prolonged (i.e., ≥10 y) multivitamin or supplement use compared with nonuse. However, it is well recognized that the potential benefits of multivitamin use may be confounded by particular health, diet or lifestyle factors (68,69) and individuals who routinely use multivitamins for long periods may be more likely to have healthier lifestyles. These factors may confound the results of observational studies because they may individually or in combination contribute to cancer risk reduction.

The effect of folate intake and multivitamin use on relative risk of colon cancer according to family history of colorectal cancer in a first-degree relative was evaluated in a large (n = 88,758) prospective cohort study of women (70). The observed inverse association between folic acid intake and colon cancer risk was greatest in women with a family history of the disease. Colon cancer risk was reduced by 52% in women with a family history who consumed >400 μg/d compared with women with a similar family history who consumed ≤200 μg/d. In contrast, in women without a family history, the protective effect of folic acid was much less dramatic. Use of multivitamins for >5 y substantially attenuated the risk associated with a family history of colorectal cancer whereas duration of multivitamin use had no effect on risk in women without a family history of the disease. Moderate-to-heavy alcohol consumption increased the risk associated with family history. The results suggest that regular use of multivitamins (>5 y) and avoidance of moderate-to-heavy alcohol consumption may diminish the excess risk of colon cancer associated with a family history of the disease.

Data from large epidemiologic studies such as the Nurses' Health Study (11) and the Canadian National Breast Screening Study (71) suggest that increased risk of breast cancer associated with regular alcohol consumption [≥14–15 g/d, equivalent to ~5–6 oz (148–177 mL) of wine and 13–14 oz (384–414 mL) of beer] may be reduced by adequate folate intake. In the Nurses' Health Study (11), among women who consumed ≥15 g/d of alcohol, risk for breast cancer was lowest in those consuming ≥600 μg/d of folate from food and supplements (multivariate-adjusted relative risk = 0.56) compared with women consuming 150–299 μg/d of folate. Women consuming alcohol and taking a multivitamin supplement had 26% reduced risk for breast cancer compared with nonusers of supplements. Similarly, in the Canadian National Breast Screening Study (71), women consuming ≥14 g/d of alcohol and >300 μg/d of folate had a 43% decreased rate of breast cancer compared with women with the same alcoholic intake and consuming <225 μg/d of folate. In this study, disease rate ratios were based on folate intake from food only because data were not available for multivitamin supplement use. Folate status in chronic alcohol users may be negatively impacted by low intake, decreased absorption or altered metabolism of folate (72). Higher folate intakes may compensate for the negative influence of high alcohol consumption on folate metabolism and may translate into the reduced breast cancer risks observed in these studies.

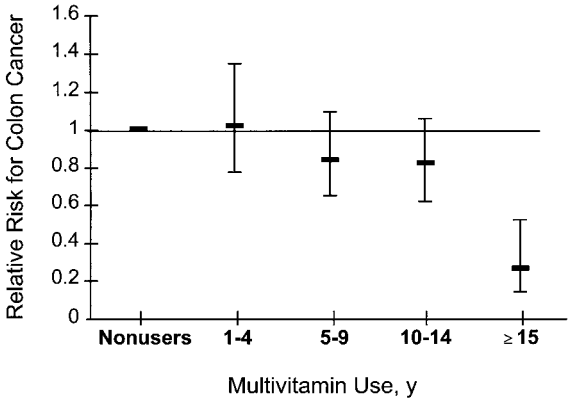

FIGURE 7 Relative risk and 95% confidence intervals for colon cancer based on duration of use of multivitamins containing folic acid. Reprinted from Giovannucci et al. (9) with permission.

Epidemiologic studies using large cohort groups support an inverse association between folate and risk of colorectal dysplasia or neoplasia. However, the evidence does not yet support public health recommendations regarding folic acid and prevention of colon cancer. Currently, there are four large-scale randomized placebo-controlled intervention trials ongoing in the United States to evaluate the efficacy of supplemental folic acid in the prevention of colon cancer (73). Thorough evaluation of the results of these studies should provide a better understanding of the potential role of folate in colon cancer prevention.

Summary

Folic acid supplements reduce NTD rates when taken periconceptionally and are associated with reduced risk of vascular disease and colon cancer in observational studies. The strength of the scientific evidence regarding the effectiveness of folic acid supplements to reduce NTD has led to public health recommendations to take folic acid supplements periconceptionally. This recommendation related to folic acid supplement use was coupled with the implementation of mandatory folic acid fortification in the United States and Canada. Data suggest that consumption of foods fortified with folic acid and related improvements in folate status have been associated with a reduction in NTD. The reduction in NTD rates has not been attributed to changes in periconceptional folic acid supplement use, which with a few exceptions has not increased in response to public health recommendations. The apparent positive reduction in NTD occurrence that has been associated with enhanced folate status postfortification supports food fortification as an effective intervention strategy. This approach, however, is not universally accepted, as illustrated by the fact that few countries other than the United States and Canada have mandated folic acid fortification of foods. Educational efforts to increase periconceptional folic acid supplement use should continue with the recognition, based on conclusions from intervention trials, that compliance with recommendations will result in reduced NTD incidence.

The potential for folic acid supplements to reduce chronic disease risk has enormous implications for public health because of the large percentage of the population likely to be affected. The complexity of disease processes such as vascular disease and cancer have compromised the ability of researchers to reach definitive conclusions regarding the efficacy of folic acid supplementation and disease risk. Data from population-based studies have provided the impetus for on-going intervention trials designed to determine whether folic acid sup-

plementation results in a reduction of disease incidence and morbidity. Promising data related to the inverse association between folic acid intake and clinical markers for vascular disease provide the basis for continued investigations. The influence of alcohol consumption on cancer risk when coupled with low folate intake illustrates the multifactorial nature of chronic disease and the challenge of interpreting research findings related to folic acid supplementation and disease risk.

LITERATURE CITED

1. Bailey, L. B., Moyers, S. & Gregory, J. F., III. (2001) Folate. In: Present Knowledge in Nutrition, 8th ed. (Bowman, B. A. & Russell, R. M., eds). ILSI Press, Washington, DC.

2. Berry, R. J., Li, Z., Erickson, J. D., Li, S., Moore, C. A., Wang, H., Mulinare, J., Zhao, P., Wong, L-Y. C., Gindler, J., Hong, S-X. & Correa, A. (1999) Prevention of neural-tube defects with folic acid in China. N. Engl. J. Med. 341: 1485–1490.

3. Czeizel, A. E. & Dudas, I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N. Engl. J. Med. 327: 1832–1835.

4. MRC Vitamin Study Research Group. (1991) Prevention of neural tube defects: results of the Medical Research Council vitamin study. Lancet 338: 131–137.

5. Centers for Disease Control and Prevention. (1992) Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR 41: 1–7.

6. Institute of Medicine. (1998) Folate. In: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. pp. 196–305. National Academy Press, Washington, DC.

7. Boushey, C. J, Beresford, S.A.A., Omenn, G. S. & Motulsky, A. G. (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA 274: 1049–1057.

8. Eikelboom, J. W., Lonn, E., Genest, J., Hankey, G. & Yusuf, S. (1999) Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann. Intern. Med. 131: 363–375.

9. Giovannucci, E., Stampfer, M. J., Colditz, G. A., Hunter, D. J., Fuchs, C., Rosner, B. A., Speizer, F. E. & Willett, W. C. (1998) Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann. Intern. Med. 129: 517–524.

10. Sellers, T. A., Kushi, L. H., Cerhan, J. R., Vierkant, R. A., Gapstur, S. M., Vachon, C. M., Olson, J. E., Therneau, T. M. & Folsom, A. R. (2001) Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 12: 420–428.

11. Zhang, S., Hunter, D. J., Hankinson, S. E., Giovannucci, E. L., Rosner, B. A., Colditz, G. A., Speizer, F. E. & Willett, W. C. (1999) A prospective study of folate intake and the risk of breast cancer. JAMA 281: 1632–1637.

12. Botto, L. D., Moore, C. A., Khoury, M. J. & Erickson, J. D. (1999) Neural-tube defects. N. Engl. J. Med. 341: 1509–1519.

13. Food and Drug Administration. (1996) Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Final rule. 21 CFR Parts 136, 137, and 139. pp. 8781–8807.

14. Health Canada. (1997) Regulations amending the Food and Drug Regulations (1066). Canada Gazette, Part I. 131: 3702–3737.

15. Lumley, J., Watson, L., Watson, M. & Bower, C. (2001) Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects (Cochrane Review). Cochrane Database Syst. Rev. 3: CD001056.

16. Institute of Medicine. (2002) Evolution of evidence for selected nutrient and disease relationships. National Academy Press, Washington, DC.

17. Bower, C. & Stanley, F. J. (1989) Dietary folate as a risk factor for neural-tube defects: evidence from a case-control study in Western Australia. Med. J. Aust. 150: 613–619.

18. Laurence, K. M., James, N., Miller, M. H., Tennant, G. B. & Campbell, H. (1981) Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. Br. Med. J. 282: 1509–1511.

19. Milunsky, A., Jick, H., Jick, S. S., Bruell, C. L., MacLaughlin, D. S., Rothman, K. J. & Willett, W. (1989) Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA 262: 2847–2852.

20. Mulinare, J., Cordero, J. F., Erickson, J. D. & Berry, R. J. (1988) Periconceptional use of multivitamins and the occurrence of neural tube defects. JAMA 260: 3141–3145.

21. Freire, W. B., Hertrampt, E. & Cortes, F. (2000) Effect of folic acid fortification in Chile: preliminary results. Eur. J. Pediatr. Surg. 10: 42–43.

22. Centers for Disease Control and Prevention. (2000) Folate status in women of childbearing age—United States, 1999. MMWR 49: 962–965.

23. Choumenkovitch, S. F., Jacques, P. J., Nadeau, M. R., Wilson, P.W.F., Rosenberg, I. H. & Selhub, J. (2001) Folic acid fortification increases red blood cell folate concentrations in the Framingham Study. J. Nutr. 131: 3277–3280.

24. Jacques, P. F., Selhub, J., Bostom, A. G., Wilson, P.W.F. & Rosenberg, I. H. (1999) The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N. Engl. J. Med. 340: 1449–1454.

25. Lawrence, J. M., Petitti, D. B., Watkins, M. & Umekubo, M. A. (1999) Trends in serum folate after food fortification. Lancet 354: 915–916.

26. Ray, J. G., Vermeulen, M. J., Boss, S. C. & Cole, D.E.C. (2002) Increased red cell folate concentrations in women of reproductive age after Canadian folic acid food fortification. Epidemiology 13: 238–240.

27. Metz, J., Sikaris, K. A., Maxwell, E. L. & Levin, M. D. (2002) Changes in serum folate concentrations following voluntary food fortification in Australia. Med. J. Aust. 176: 90–91.

28. Honein, M. A., Paulozzi, L. J., Mathews, T. J., Erickson, J. D. & Wong, L-Y.C. (2001) Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA 285: 2981–2986.

29. Williams, L. J., Mai, C. T., Edmonds, L. D., Shaw, G. M., Kirby, R. S., Hobbs, C. A., Sever, L. E., Miller, L. A., Meaney, F. J. & Levitt, M. (2002) Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. Teratology 66: 33–39.

30. Gucciardi, E., Pietrusiak, M-A., Reynolds, D. L. & Rouleau, J. (2002) Incidence of neural tube defects in Ontario, 1986–1999. Can. Med. Assoc. J. 167: 237–240.

31. Persad, V. L., Van den Hof, M. C., Dube, J. M. & Zimmer, P. (2002) Incidence of open neural tube defects in Nova Scotia after folic acid fortification. Can. Med. Assoc. J.167: 241–245.

32. Bower, C., Ryan, A., Rudy, E. & Miller, M. (2002) Trends in neural tube defects in Western Australia. Aust. N. Z. J. Public Health 26: 150–151.

33. March of Dimes Birth Defects Foundation. (2002) Folic acid and the prevention of birth defects. A national survey of pre-pregnancy awareness and behavior among women of childbearing age 1995–2002. March of Dimes Birth Defects Foundation, White Plains, NY.

34. De La Vega, A., Salicrup, E. & Verdiales, M. (2002) A nationwide program for the use of preconceptional folic acid to prevent the development of open neural tube defects. Who is really using folic acid? Puerto Rico Health Sci. J. 21: 7–9.

35. De Walle, H.E.K. & de Jong-van den Berg, L.T.W. (2002) Insufficient folic acid intake in the Netherlands: what about the future? Teratology 66: 40–43.

36. Miller, M., Bower, C., Payne, J. & Serna, P. (2000) Folate-related knowledge and practices of Western Australian women before and during pregnancy Teratology 62: 367(abs).

37. Stevenson, R. E., Allen, W. P., Pai, G. S., Best, R., Seaver, L. H., Dean, J. & Thompson, S. (2000) Decline in prevalence of neural tube defects in a high-risk region of the United States. Pediatrics 106: 677–683.

38. Rader, J. I. & Yetley, E. A. (2002) Nationwide folate fortification has complex ramifications and requires careful monitoring over time. Arch. Intern. Med. 162: 608–609.

39. Oakley, G. P. (2002) Delaying folic acid fortification of flour. BMJ 324: 1348–1349.

40. Oakley, G. P. (2002) Inertia on folic acid fortification: public health malpractice. Teratology 66: 44–54.

41. Baik, H. W. & Russell, R. M. (1999) Vitamin B12 deficiency in the elderly. Annu. Rev. Nutr. 19: 357–377.

42. Department of Health (2000) Folic Acid and the Prevention of Disease. Report on Health and Social Subjects no. 50, London: The Stationery Office.

43. Food Standards Agency. (2002) Board reaches conclusion on folic acid. Available at http://www.foodstandards.gov.uk/news/newsarchive/62488. Accessed August 1.

44. Postma, M. J., Londeman, J., Veenstra, M., de Walle, H.E.K. & de Jong-van den Berg, L.T.W. (2002) Cost-effectiveness of periconceptional supplementation of folic acid. Pharm. World Sci. 24: 8–11.

45. Refsum, H., Ueland, P. M., Nygard, O. & Vollset, S. E. (1998) Homocysteine and cardiovascular disease. Annu. Rev. Med. 49: 31–62.

46. Christen, W. G., Ajani, U. A., Glynn, R. J. & Hennekens, C. H. (2000) Blood levels of homocysteine and increased risks of cardiovascular disease. Causal or casual? Arch. Intern. Med. 160: 422–434.

47. Homocysteine Lowering Trialists' Collaboration. (1998) Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. BMJ 316: 894–898.

48. Brouwer, I. A., van Dusseldorp, M., Thomas, C.M.G., Duran, M., Hautvast, J.G.A.J., Eskes, T.K.A.B. & Steegers-Theunissen, R.P.M. (1999) Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. Am. J. Clin. Nutr. 69: 99–104.

49. Bronstrup, A., Hages, M., Prinz-Langenohl, R. & Pietrzik, K. (1998) Effects of folic acid and combinations of folic acid and vitamin B-12 on plasma homocysteine concentrations in healthy, young women. Am. J. Clin. Nutr. 68: 1104–1110.

50. de Bree, A., Verschuren, W.M.M., Blom, H. J. & Kromhout, D. (2001) Association between B vitamin intake and plasma homocysteine concentration in the general Dutch population aged 20–65 y. Am. J. Clin. Nutr. 73: 1027–1033.

51. Christodoulakos, G., Panoulis, C., Rizos, D., Moustakarias, T., Phocas, I. & Creatsas, G. (2001) Homocysteine and folate levels in postmenopausal women. Maturitas 39: 161–167.

52. Knekt, P., Alfthan, G., Aromaa, A., Heliovaara, M., Marniemi, J., Rissanen, H. & Reunanen, A. (2001) Homocysteine and major coronary events: a prospective population study amongst women. J. Intern. Med. 249: 461–465.

53. Doshi, S. N., McDowell, I.F.W., Moat, S. J., Payne, N., Durrant, H. J., Lewis, M. J. & Goodfellow, J. (2002) Folic acid improves endothelial function

in coronary artery disease via mechanisms largely independent of homocysteine lowering. Circulation 105: 22–26.

54. Title, L. M., Cummings, P. M., Giddens, K., Genest, J. J. & Nassar, B. A. (2000) Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. J. Am. Coll. Cardiol. 36: 758–765.

55. Verhaar, M. C., Wever, R.M.F., Kastelein, J.J.P., van Loon, D., Milstien, S., Koomans, H. A. & Rabelink, T. J. (1999) Effects of oral folic acid supplementation on endothelial function in familial hypercholesterolemia. Circulation 100: 335–338.

56. Woo, K. S., Chook, P., Lolin, Y. I., Sanderson, J. E., Metreweli, C. & Celermajer, D. S. (1999) Folic acid improves arterial endothelial function in adults with hyperhomocystinemia. J. Am. Coll. Cardiol. 34: 2002–2006.

57. Vermeulen, E.G.J., Stehouwer, C.D.A., Twisk, J.W.R., van den Berg, M., de Jong, S. C., Mackaay, A.J.C., van Campen, C.M.C., Visser, F. C., Jakobs, C.A.J.M., Bulterijs, E. J. & Rauwerda, J. A. (2000) Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomised, placebo-controlled trial. Lancet 355: 517–522.

58. Schnyder, G., Roffi, M., Flammer, Y., Pin, R. & Hess, O. M. (2002) Effect of homocysteine-lowering therapy with folic acid, vitamin $B_{12,}$ and vitamin B_6 on clinical outcome after percutaneous coronary intervention The Swiss Heart Study: A Randomized Controlled Trial. JAMA 288: 973–979.

59. Bostom, A. G., Selhub, J., Jacques, P. F. & Rosenberg, I. H. (2001) Power shortage: clinical trials testing the "Homocsyteine Hypothesis" against a background of folic acid-fortified cereal grain flour. Ann. Intern. Med. 135: 133–137.

60. Choi, S-W. & Mason, J. B. (2000) Folate and carcinogenesis: an integrated scheme. J. Nutr. 130: 129–132.

61. Cravo, M., Fidalgo, P., Pereira, A. D., Gouveia-Oliveira, A., Chaves, P., Selhub, J., Mason, J. B., Mira, F. C. & Leitao, C. N. (1994) DNA methylation as an intermediate biomarker in colorectal cancer: modulation by folic acid supplementation. Eur. J. Cancer Prev. 3: 473–479.

62. Cravo, M. L., Pinto, A. G., Chaves, P., Cruz, J. A., Lage, P., Leitao, C. N. & Mira, F. C. (1998) Effect of folate supplementation on DNA methylation of rectal mucosa in patients with colonic adenomas: correlation with nutrient intake. Clin. Nutr. 17: 45–49.

63. Khosraviani, K., Weir, H. P., Hamilton, P., Moorehead, J. & Williamson, K. (2002) Effect of folate supplementation on mucosal cell proliferation in high risk patients for colon cancer. Gut 51: 195–199.

64. Rampersaud, G. C., Bailey, L. B. & Kauwell, G.P.A. (2002) Relationship of folate to colorectal and cervical cancer: review and recommendations for practitioners. J. Am. Diet. Assoc. 102: 1273–1282.

65. Giovannucci, E., Stampfer, M. J., Colditz, G. A., Rimm, E. B., Trichopoulos, D., Rosner, B. A., Speizer, F. E. & Willett, W. C. (1993) Folate, methionine, and alcohol intake and risk of colorectal adenoma. J. Natl. Cancer Inst. 85: 875–884.

66. White, E., Shannon, J. S. & Patterson, R. E. (1997) Relationship between vitamin and calcium supplement use and colon cancer. Cancer Epidemiol. Biomarkers Prev. 6: 769–774.

67. Zeigler, R. G., Jones, C. J., Brinton, L. A., Norman, S. A., Mallin, K., Levine, R. S., Lehman, H. F., Hamman, R. F., Trumble, A. C., Rosenthal, J. F. & Hoover, R. N. (1991) Diet and the risk of in situ cervical cancer among white women in the United States. Cancer Causes Control 2: 17–29.

68. Lyle, B. J., Mares-Perlman, J. A., Klein, B.E.K., Klein, R. & Greger, J. L. (1998) Supplement users differ from nonusers in demographic, lifestyle, dietary and health characteristics. J. Nutr. 128: 2355–2362.

69. Patterson, R. E., Neuhouser, M. L., White, E., Hunt, J. R. & Kristal, A. R. (1998) Cancer-related behavior of vitamin supplement users. Cancer Epidemiol. Biomarkers. Prev. 7: 79–81.

70. Fuchs, C. S., Willett, W. C., Colditz, G. A., Hunter, D. J., Stampfer, M. J., Speizer, F. E. & Giovannucci, E. L. (2002) The influence of folate and multivitamin use on the familial risk of colon cancer in women. Cancer Epidemiol. Biomarkers. Prev. 11: 227–234.

71. Rohan, T. E., Jain, M., Howe, G. R. & Miller, A. B. (2000) Dietary folate consumption and breast cancer risk. J. Natl. Cancer Inst. 92: 266–269.

72. Halstead, C. H., Villanueva, J. A., Devlin, A. M. & Chandler, C. J. (2002) Metabolic interactions of alcohol and folate. J. Nutr. 132: 2367S–2372S.

73. Kim, Y-I. (1999) Folate and cancer prevention: a new medical application of folate beyond hyperhomocysteinemia and neural tube defects. Nutr. Rev. 57: 314–321.