Brief Critical Review

August 1999: 250–260

Vitamin B_{12} Deficiency: A New Risk Factor for Breast Cancer?

A prospective epidemiologic study found a threshold level for serum vitamin B_{12}, below which an increased risk of breast cancer among postmenopausal women was observed. This is the first observation to suggest that B_{12} status may influence breast carcinogenesis and therefore may be a modifiable risk factor for breast cancer prevention.

Breast cancer is the most common cancer in women. It is estimated that 176,300 new cases of breast cancer will be diagnosed in 1999 in the United States and 43,700 deaths will be directly attributable to the cancer.[1] The magnitude of the problem is similar in most developed nations. Breast cancer strikes women of all ages, races, ethnicities, socioeconomic strata, and geographic locales. Until recently, the incidence of breast cancer appeared to be rising. This trend held true, even when corrected for an increased detection rate owing to improved technology and heightened public awareness. Recent data from the National Cancer Institute now show a decline in breast cancer mortality, suggesting that earlier detection and/or improvements in treatment may be making the prognosis somewhat more favorable.

Breast cancer is a heterogenous disease whose etiology for the most part is unknown.[2] However, it is generally agreed that there are three broad determinants of breast cancer risk. One determinant is heredity, such as carrying a germline mutation in the BRCA1 or BRCA2 gene. Another determinant is hormonal and reproductive factors. Women have a 180-fold higher risk of breast cancer than men, and among women, age at menarche, age at menopause, age at first childbirth, and parity influence breast cancer risk. A third determinant is environment, such as socioeconomic status, lifestyle habits, and diet.

Many dietary components have been evaluated in epidemiologic or animal studies for their influence on breast cancer risk. One of the first dietary components to be implicated in the high incidence of breast cancer was dietary fat.[3] Correlations observed between breast cancer rates and per capita animal fat consumption in different countries have been followed by studies on the association between individual fat consumption and breast cancer risk in women. However, prospective studies have failed to show a clear association between a woman's daily total fat intake and breast cancer risk. Alcohol also is a well-established dietary risk factor in breast cancer. Studies with alcohol have shown that moderate consumption increases breast cancer risk by approximately 10%, reportedly by altering estrogen metabolism.

On the other hand, most case-control studies have suggested that a higher intake of fiber, vegetables, and fruits protect against breast cancer. Vegetables and fruits are rich in fiber, minerals, anticarcinogenic phytochemicals, and antioxidant vitamins. A high fiber intake is thought to reduce estrogen levels by decreasing absorption of estradiol formed in the colon, and several phytochemicals have shown a protective effect in experimental models of mammary gland carcinogenesis. Numerous epidemiologic studies have investigated antioxidant vitamins such as ß-carotene, vitamin C, and vitamin E for a protective effect against breast cancer. The results of case-control and prospective studies, however, are unconvincing.[3]

The recent report by Wu et al. suggests a relationship between serum vitamin B_{12} concentrations and breast cancer risk.[4] They conducted a nested case-control study using resources from the Washington County (Maryland) serum bank to investigate the incidence of breast cancer and prediagnostic serum levels of folate, B_{12}, and vitamin B_6. Among postmenopausal women at blood donation, observed associations of B_{12} and breast cancer suggested a threshold effect: an increased risk of cancer was observed in the quintile of subjects who possessed the lowest B_{12} levels compared with the higher four-fifths of the control distribution. On the other hand, the investigators found no evidence of an association between breast cancer incidence and blood levels of folate, vitamin B_6, or homocysteine, all of which, like B_{12}, are involved in one-carbon metabolism.

To my knowledge, this is the first time a relationship between blood levels of B_{12} and breast cancer incidence has been examined. As is true of most observational studies, it is not known whether the low B_{12} status in these subjects is a factor that enhances breast cancer develop-

This review was prepared by Sang-Woon Choi, M.D., Ph.D., Vitamin Bioavailability Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111, USA.

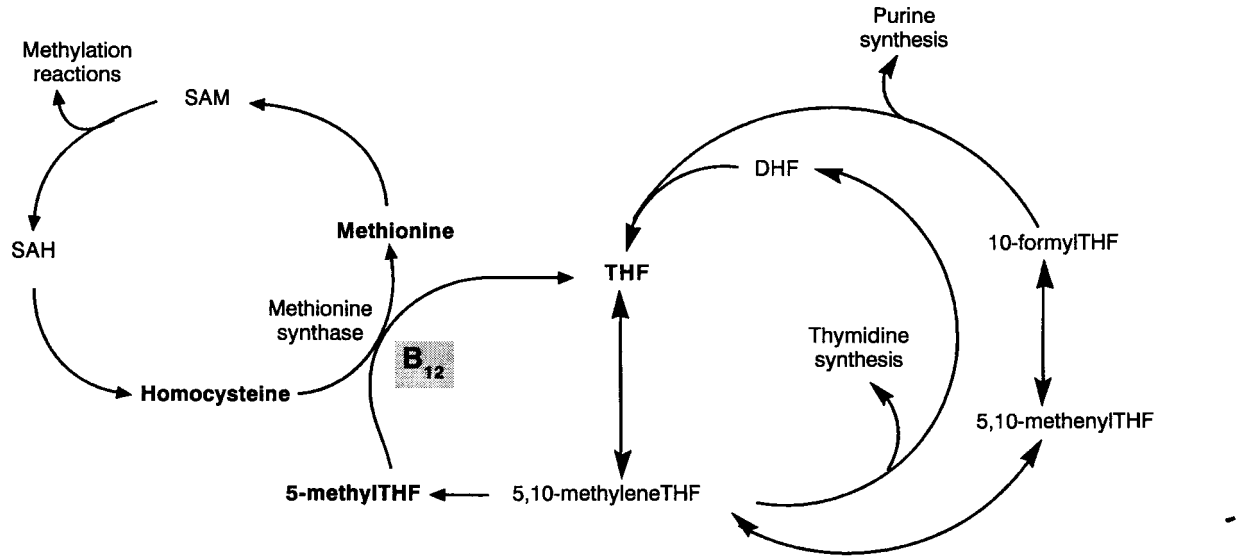

Figure 1. The interrelationships of vitamin B_{12}, folate, and methionine. THF = tetrahydrofolate; DHF = dihydrofolate; SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine.

ment or whether it is a natural consequence of breast cancer.

Vitamin B_{12} (cobalamin) has been a challenging problem in biochemistry and medicine since the discovery by George Minot and William Murphy in 1926 that pernicious anemia could be treated by feeding the patient large amounts of liver. A possible functional relationship between B_{12} and cancer was established in 1954, when Massey and Rubin described the persistence of abnormal gastric columnar cells in the stomachs of individuals with pernicious anemia, even after the anemia had been successfully treated with B_{12}.[5] They postulated that these abnormal cells might represent a transitional cell type between cells that characterize the atrophic gastritis epithelium in pernicious anemia and gastric cancer cells. Two prospective clinical intervention trials subsequently demonstrated a significant degree of attenuation in bronchial metaplasia after supplementation with B_{12} and folate.[6,7] In 1986 Herbert[8] suggested that the role of B_{12} in preventing carcinogenesis is largely an extension of, and linked to, its roles in normal metabolism, particularly one-carbon unit metabolism. A possible key function in this regard may be its role in DNA methylation whereby hypomethylation "switches on" genes and methylation "switches them off."[8]

In mammalian tissue, B_{12} is involved in two types of reactions as a coenzyme: 1) rearrangements, exemplified by the conversion of L-methylmalonyl CoA into succinyl CoA, which is the point of entry for some of the carbon atoms of methionine, isoleucine, threonine, and valine into the Kreb's cycle and 2) biological methylation, as in the synthesis of methionine.[9] A B_{12}-containing enzyme removes a methyl group from methyl folate and delivers it to homocysteine, thereby converting homocysteine to methionine and regenerating tetrahydrofolate, from which 5,10-methylene tetrahydrofolate (necessary for thymidylate synthesis) is made (Figure 1). Because methyl folate may only return to the body's folate pool via a B_{12}-dependent step, a patient with B_{12} deficiency has much of his folate "trapped" as methyl folate, a metabolically inactive form as far as nucleotide synthesis is concerned. This "folate trap" hypothesis helps explain why the hematologic damage of B_{12} deficiency is not clinically distinguishable from that of folate deficiency. In either deficiency, lack of adequate DNA synthesis causes many hemopoietic cells to die in the bone marrow and induces megaloblastosis, or so-called "ineffective erythropoiesis." Megaloblastosis is also present in all other rapidly duplicating cells of the body, such as in the gastrointestinal epithelium.

The features of B_{12} metabolism suggest two possible mechanisms by which B_{12} depletion might enhance carcinogenesis, and these are quite similar to folate-related carcinogenesis.[10] One potential mechanism is an increase in DNA strand breaks. A folate trap produced by B_{12} deficiency decreases thymidine and purine synthesis and subsequently induces a deoxyribonucleotide pool imbalance. This imbalance causes uracil to be incorporated into DNA instead of thymidine. Uracil in DNA is excised by a repair glycosylase with the formation of a transient single-strand break in the DNA; two opposing single-strand breaks cause a double-strand chromosomal break, which is difficult to repair and which may ultimately turn into a mutation. In a study of healthy elderly men or young adults, increased chromosome breakage correlated either with a deficiency of folate or B_{12} or with elevated levels of homocysteine; dietary supplementation of B_{12} above RDA level minimized this effect.[11]

The other possible mechanism is an alteration of DNA methylation by the methyl folate trap.[8] Mammalian DNA is methylated at deoxycytidine residues. Nearly all of these methylated residues reside in cytosine-guanine (*5′-CpG-3′*) sequences, and 70–90% of the deoxycytosines in *CpG* sequences are methylated (which accounts for 3–5 % of deoxycytosine in DNA). Even though the entire array of functions of DNA methylation are not yet known, it is an important determinant in gene expression and structural stability of DNA.[10]

The precise means by which aberrations in DNA methylation play a role in carcinogenesis remain ill-defined, but it is clear that normal patterns of DNA methylation are necessary to maintain cellular homeostasis. Abnormal DNA methylation patterns are characteristic of neoplastic cells. Three different types of aberrant DNA methylation are associated with carcinogenesis.[12] The first is widespread areas of genomic hypomethylation. Genomic DNA hypomethylation is a common phenomenon in cancers in the colon, lung, stomach, uterus, and cervix. Recently Soares et al. reported that DNA from breast cancer also showed significant hypomethylation compared with DNA from benign lesions and from normal breast tissue.[13] The second aberrant pattern is regional hypermethylation. These changes usually occur in *CpG* dinucleotides that are clustered in regions of approximately 1–2 kb in length, called "*CpG* islands," in or near the promoter and first exon region of genes. Hypermethylation of promoter regions has been implicated in carcinogenesis because it turns off expression of these genes. Hypermethylation has been previously observed in breast cancer-related tumor suppressor genes, such as the estrogen receptor gene and the mammary-derived growth factor gene, as well as a breast cancer susceptibility gene, *BRCA1*. Aberrant cytosine methylation of the *BRCA1 CpG* island promoter is one of the candidate mechanisms of *BRCA1* repression in sporadic breast cancer.[14] The third aberrant pattern is an alteration of methylation in specific coding regions. *CpG* sites are mutational hot spots in many human tumors. Spontaneous or enzymatic deamination of 5-methyl-cytosine to thymine and enzymatic deamination of unmethylated cytosine to uracil ultimately can lead to a C to T mutation. In an animal study,[15] folate deficiency induced hypomethylation in the hypermutable exons of p53 gene, raising the question whether these might be molecular lesions that precede the mutations commonly seen in this region. In this animal experiment, DNA strand breaks also were increased in the same region. Although DNA methylation is one of the most important epigenetic changes in breast carcinogenesis and B_{12} metabolism has been known to be involved in DNA methylation, direct evidence to support this hypothesis is not yet available.

In future breast cancer studies, B_{12} depletion should be considered as a potential dietary risk factor. Mechanistic studies also would be of interest.

Another interesting observation in this study is the lack of a relationship between breast cancer incidence and plasma or serum folate level. In 1991 a case control study found that women with breast cancer had a substantially lower ingestion of dietary folate compared with controls without breast cancer, and that dietary folate intake was inversely associated with the risk of developing breast cancer.[16] In a more recent prospective study, the excess risk of breast cancer associated with alcohol consumption was reduced by adequate folate intake even though total folate intake was not associated with the overall risk of breast cancer.[17] It is well known that alcohol consumption interferes with folate metabolism, probably by changing the distribution of the different coenzyme forms of folate; this is quite similar to the effect of B_{12} deficiency. The present study, however, did not find any associations between plasma or serum folate level and breast cancer incidence or any evidence for a protective effect of B-vitamin supplementation.

We can propose several reasons why the results of epidemiologic studies of folate status and breast cancer incidence are conflicting. First, a growing body of epidemiologic and clinical evidence suggests that, regardless of systemic folate status, the susceptibility to folate depletion varies widely among tissues and may be a factor that predisposes the body to the development of neoplasms originating from these tissues. For example, in animal studies the liver is highly sensitive to low folate diets, whereas the brain is very resistant. It is not known yet whether systemic folate status directly reflects the folate concentrations in breast tissue. Second, plasma or serum folate concentration, which reflects short-term stores, is less reliable as an index for tissue folate levels compared with red blood cells (RBC) folate concentration, which reflects intermediate-term stores. Lashner et al.[18] found that the RBC folate concentration was significantly correlated with the development of dysplasia and cancer in ulcerative colitis, but serum folate concentration was not. Third, alteration of folate distribution may be more important than total folate level in the tissue. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism. A common mutation of this MTHFR gene (*C677T*) causes thermolability and reduced activity of MTHFR, and men with the homozygous mutation have half the risk of colon cancer, especially with normal folate status.[19] The product of MTHFR, 5-methyltetrahydrofolate, provides the methyl group for DNA methylation, whereas its substrate, 5,10-methylenetetrahydrofolate, is required for thymidylate and purine synthesis (Figure 1). The colon cancer protective effect of this MTHFR mutation is related to the increase of 5,10-methylenetetrahydrofolate, especially in normal folate status. This observation suggests that alteration of folate distribution may affect cancer development.

In future studies, folate level in breast tissue or, less

desirably, RBC folate concentration should be considered as the measure of folate status instead of plasma or serum folate levels.

1. Landis SH, Murray T, Bolden S, et al. Cancer statistics, 1999. CA Cancer J Clin 1999;49:8–31
2. Snyderwine EG. Diet and mammary gland carcinogenesis. In: Senn HJ, Gelber RD, Goldhirsch A, et al, eds. Recent results in cancer research. Berlin: Springer-Verlag, 1998;3–10
3. Stoll BA. Breast cancer and the Western diet: role of fatty acids and antioxidant vitamins. Eur J Cancer 1998;34:1852–6
4. Wu K, Helzlsouer KJ, Comstock GW, et al. A prospective study on folate, B_{12}, and pyridoxal 5´-phosphate (B_6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17
5. Massey BW, Rubin CE. The stomach in pernicious anemia: a cytologic study. Am J Med Sci 1954;481–92
6. Heimburger DC, Alexander CB, Birch R, et al. Improvement in bronchial squamous metaplasia in smokers treated with folate and vitamin B_{12}. Report of a preliminary randomized, double-blind intervention trial. JAMA 0988;259:1525–30
7. Saito M, Kato H, Tsuchida T, et al. Chemoprevention effects of bronchial squamous metaplasia by folate and vitamin B_{12} in heavy smokers. Chest 1994;106: 496–9
8. Herbert V. The role of vitamin B_{12} and folate in carcinogenesis. Adv Exp Med Biol 1986;206:293–311
9. 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. Malvern: Lea & Febiger, 1994;402–25
10. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem 1999;10:66–88
11. Fenech M, Aitken C, Rinaldi J. Folate, vitamin B_{12}, homocysteine status and DNA damage in young Australian adults. Carcinogenesis 1998;19:1163–71
12. Counts JL, Goodman JI. Hypomethylation of DNA: an epigenetic mechanism involved in tumor promotion. Mol Carcinog 1994;11:185–8
13. Soares J, Pinto AE, Cunha CV, et al. Global DNA hypomethylation in breast carcinoma. Correlation with prognostic factors and tumor progression. Cancer 1999;85:112–8
14. Dobrovic A, Simpfendorfer D. Methylation of the *BRCA1* gene in sporadic breast cancer. Cancer Res 1997;57:3347–50
15. Kim YI, Pogribny IP, Basnakian AG, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation within p53 tumor suppressor gene. Am J Clin Nutr 1997;65:46–52
16. Graham S, Hellmann R, Marshall J, et al. Nutritional epidemiology of postmenopausal breast cancer in western New York. Am J Epidemiol 1991;134:552–66
17. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7
18. Lashner BA, et al. Red blood cell folate is associated with the development of dyaplasia and cancer in ulcerative colitis. J Cancer Res Clin Oncol 1993;119:549–54
19. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–102

Mediterranean Diet and Coronary Heart Disease: Are Antioxidants Critical?

There is substantial evidence that several variants of the Mediterranean diet reduce the incidence of coronary heart disease (CHD) and perhaps other chronic conditions. Recently, the final results of the Lyon Diet Heart Study, a randomized secondary prevention trial, indicated that the Mediterranean diet substantially reduces the rate of recurrence after a first myocardial infarction. Data from this study also suggest that the Mediterranean diet protects against CHD through mechanisms that are independent of traditional CHD risk factors. We postulate that the antioxidant properties of several plant foods in the Mediterranean diet may be critical mediators of the beneficial effects of this diet.

This review was prepared by Antonia Trichopoulou, M.D., Effie Vasilopoulou, B.Sc., and AAreti Lagiou, M.Sc., Department of Nutrition, National School of Public Health, Athens 11521, Greece.

There are several variants of Mediterranean diet.[1] Although the diet used on the island of Crete during the 1960s has been credited with substantial health attributes by Ancel Keys and his colleagues in their celebrated ecologic study,[2] there is no analytical epidemiologic evidence documenting differential effects among the various forms of Mediterranean diet against any particular disease.

The traditional Mediterranean diet may be thought of as having eight components[3]: high monounsaturated to saturated fat ratio, high consumption of legumes, high consumption of cereals (including bread), high consumption of fruits, high consumption of vegetables, low consumption of meat and meat products, moderate consumption of milk and dairy products, and moderate ethanol consumption.

Studies during the last two decades have provided insight into the mechanisms underlying the benefits of the Mediterranean diet in relation to coronary heart disease (CHD). It has now been established that monoun-