Pooled Analyses of Prospective Cohort Studies on Folate and Colorectal cancer

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Background : Folate has been suggested to reduce risk of some cancers, especially of the colon and rectum, but no quantitative summary is available for the effect of folate on colorectal cancer risk from epidemiologic studies. Pooled analyses of individual data from the primary studies is preferable to meta-analyses of the published literature because pooling permits more standardized analyses, reduces publication bias, allows better examination of dose-response relationships, and may generate new hypotheses based on analyses of subgroups. Because prospective studies are less vulnerable to selection and recall biases that can affect case-control studies of diet-disease associations, we examined the association between folate intake and colorectal cancer risk by pooling primary data from nine prospective cohort studies that met predefined inclusion criteria. As most multivitamins contain a large amount of highly bioavailable folate compared to that consumed in typical diets, we also examined the association between multivitamin use and colorectal cancer risk.

Methods : We pooled the primary data from nine prospective cohort studies (Alpha-Tocopherol Beta-Carotene Cancer Prevention Study, Canadian National Breast Screening Study, Health Professionals Follow-up Study, Iowa Women's Health Study, Netherlands Cohort Study, New York State Cohort, New York University Women's Health Study, Nurses' Health Study, and Sweden Mammography Cohort) in North America and Europe that met predefined criteria. The main exposure variables and other covariates were defined in a standardized manner across studies. Diet was assessed at baseline in each study with a study-specific food frequency questionnaire. We obtained intake data for folate from foods (dietary folate) and for folate from foods and supplements (total folate), if available, from each study. Dietary and total folate, as well as other nutrient intakes, were energy-adjusted by the residual method. Each study was analyzed by a method consistent with its study design. The Canadian National Breast Screening Study and the Netherlands Cohort Study were analyzed as case-cohort studies using Epicure software. The other

studies were analyzed using the Cox proportional hazards model, and incidence rate ratios were estimated using SAS PROC PHREG. The study-specific estimates were combined using a random-effects model.

Results : A total of 4,824 individuals were diagnosed with incident colorectal cancers over up to 7-14 years of follow-up among the 163,302 men and 339,635 women in the nine cohort studies. Both dietary and total folate intakes were inversely associated with colorectal cancer risk. The pooled multivariate-adjusted relative risk for comparison of the highest vs lowest quintile of dietary folate intake was 0.91 (95 percent confidence interval [95% CI] 0.83 to 1.00, p for trend = 0.08). There was no evidence of heterogeneity between studies (p for heterogeneity = 0.97) or by sex (p for heterogeneity = 0.82). When we examined the association for dietary folate intake among individuals who did not use multivitamins, the inverse association persisted but was not statistically significant (pooled multivariate RR for highest vs lowest quintile = 0.89 [95% CI 0.79-1.01], p for trend = 0.09). A stronger association was observed for total folate which included intake from both foods and supplements (pooled multivariate RR for highest vs lowest quintile = 0.79 [95% CI 0.70-0.89], p for trend = 0.001, test for between-studies heterogeneity = 0.51, test for between-studies heterogeneity by sex = 0.81). The inverse association was observed among both men and women. In the analysis of total folate as a continuous variable, a 14% risk reduction (95% CI 4%-23%) was estimated for every 400-μg/day increase in total folate. The associations for both dietary and total folate intake were unchanged after we excluded cases diagnosed during the first 2 years of follow-up. The relative risks for comparisons of the highest vs lowest quintile of total folate intake were not materially changed after further adjustment for intake of total vitamins A, C, D, and E, dietary β-carotene, total calcium, methionine, and dietary fiber. To reduce the possibility of residual confounding by inadequate control for the effect of other micronutrients hypothesized to be inversely related to colorectal cancer risk, we examined associations with total folate intake in subgroups restricted to the highest quintiles of intake of these micronutrients and adding continuous terms for them. The pattern of the association between total folate consumption and colorectal cancer risk was similar.

Since the statistically significant, inverse association was observed in the highest two quintiles of total folate intake, which mostly consists of individuals who are taking multivitamins, we examined the association between multivitamin use and colorectal cancer risk. In the comparison of multivitamin users and non-users, those who were taking multivitamins regularly at baseline had a 18% lower risk of colorectal cancer (pooled multivariate RR = 0.82 [95% CI 0.72-0.92]), after adjusting for potential covariates. We also conducted analyses using categories based on identical intake cutpoints for total folate intakes in each study, based on multiples of 80 μg above the average U.S. intake of approximately 240 μg/day. The optimal folate intake appeared to be greater than the current Recommended Dietary Allowance (RDA) of

400 μg/day, but intakes beyond 600 μg/day were not associated with further reductions in risk. There was no significant difference in the effect of total folate intake according to subsite in the large bowel. In the decile analyses, however, there was a suggestion that the inverse association with total folate intake was stronger for cancers of the distal colon than for those of the proximal colon (pooled multivariate RR, 0.86 [95% CI 0.65-1.13] for proximal colon, and 0.71 [95% CI 0.53-0.95] for distal colon, p-value, test for between-studies heterogeneity by subsite = 0.35).

Discussion : Folate intake assessed by food frequency questionnaires was strongly correlated with intake measured by dietary records or multiple 24-hour recalls, with correlation coefficients ranging from 0.43 to 0.77 in the studies included in our analyses. In addition, the correlation between folate intake assessed by food frequency questionnaire and erythrocyte folate level, regarded as a good indicator of body stores of folate, was 0.56 in the Health Professionals Follow-up Study and 0.55 in the Nurses' Health Study. Thus, folate levels measured in these cohorts appear to agree with other measures of intake. However, because questionnaire estimation of folate intake is inevitably less than perfect, the magnitude of the inverse associations with colorectal cancer risk that we observed are likely to have been underestimated.

Consumption of vegetables, one of the major contributors of dietary folate intake, and multivitamin use have been shown to be correlated with other lifestyle factors that may also be associated with colorectal cancer risk. In this analysis, however, the multivariate RR, after adjustment for potential covariates simultaneously, was minimally attenuated from the age-adjusted RR for dietary and total folate intake, suggesting that residual confounding by other lifestyle factors is not likely to confound the association appreciably. The observed association with folate intake did not appear to be due to other micronutrients contained in multivitamins. First, there was an inverse association between dietary folate and colorectal cancer risk even among multivitamin non-users. Second, the inverse association with total folate intake persisted even after adjustment for intakes of total vitamins A, C, D, and E, and total calcium. Lastly, the inverse association with folate was observed among participants in the highest quintile of intake of these other micronutrients. The association with multivitamin use also suggests that the inverse association with folate from dietary sources is not due to other phytochemicals in foods.

Folate is critical for the synthesis and regeneration of S-adenosylmethionine, which serves as the essential methyl donor for over 100 biochemical reactions, including the methylation of DNA. Consequently, low plasma folate levels may lead to global hypomethylation of DNA, an early event in colorectal carcinogenesis. Low folate intake can also cause misincorporation of uracil during DNA synthesis, which can lead to DNA double-strand breaks, that in turn cause chromosome aberrations and neoplastic transformation. Recent reports of an association between a functional polymorphism in the

methyltetrahydrofolate reductase gene and colorectal cancer risk further support a specific role of folate in colorectal carcinogenesis.

In summary, we observed a modest, inverse association between folate intake and risk of colorectal cancer. These findings were consistent across studies and among men and women. A stronger inverse association was observed for the top two quintiles of total folate, which was largely related to a lower risk among users of multivitamin supplements. Although we cannot completely exclude the possibility that other constituents of multivitamins contribute to lower risk of colorectal cancer, our findings support the hypothesis that high folic acid intake reduce the risk of colorectal cancer.