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Fruit, Vegetables, and Folate: Cultivating the Evidence for Cancer Prevention

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Since the 1980s, the concept that a diet high in fruits and vegetables could reduce the risk of cancer has been promoted widely.[1] A role for diet in preventing colon cancer held particular appeal in view of the direct contact of the colonic mucosa with nutrients and toxins from foods.[2] Several mechanisms were suggested to account for these putative anti-neoplastic properties. Fiber derived from fruits, vegetables and grains was proposed to dilute or adsorb fecal carcinogens, modulate colonic transit time, alter bile acid metabolism, reduce colonic pH, or increase the production of short-chain fatty acids.[3] Fruits and vegetables are also rich sources of micronutrients, including folate, which have been actively investigated as chemopreventive agents. More recently, emerging evidence has underscored the importance of the interplay between diet and the microbial ecology of the colon.[4]

Based upon early human findings linking high intake of fruits and vegetables with lower risk of colon cancer,[5] the National Cancer Institute launched the 5-A-Day Program in 1991 with the goal of increasing the average U.S. consumption of fruits and vegetables to 5 or more servings a day. However, initial human evidence was derived largely from case-control studies, and results from prospective cohorts began to emerge that showed nonexistent or weak associations,[6, 7] including a pooled analysis of 14 studies.[8] Thus, an international expert panel convened in 2007 concluded that there was limited evidence to support an inverse association between fruits or vegetable intake and colorectal cancer, reversing their earlier opinion from 10 years prior that evidence of benefit was convincing.[9]

The obvious explanation for the inconsistency between the largely positive results from case-control studies and the disappointing null findings of cohort studies is that case-control studies, in which most dietary information is collected after diagnosis of cancer, are more prone to bias. Generally, individuals with cancer are more likely to recall perceived unhealthy dietary behaviors compared with healthy control subjects, whose willingness to participate in studies may be associated more health conscious behaviors. However, methodological bias does not tell the entire story. Recent analyses of large cohorts have also reached different conclusions from prior prospective studies, largely agreeing with the positive case-control studies. Most notably, the European Prospective Investigation Into Cancer and Nutrition (EPIC) study conducted among 500,000 individuals from 10 European

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populations, did demonstrate a 25% reduced risk of colorectal cancer among individuals with the highest intake of fiber or fruits and vegetables.[10, 11]

So what might explain these disparate results? In this issue of *Gastroenterology*, two studies delve more deeply into the relationship between fruits, vegetables, folate, and colorectal cancer and suggest that potential associations are more complex than initially considered. Specifically, assessing human evidence for an association requires more than a simple comparison of disease incidence among populations with high versus low intake of fruits, vegetables, and folate. Rather, studies should carefully consider the specifics of *how* the diet-cancer relationship is analyzed, *where* associations are examined, both in terms of anatomic subsites of the colorectum and the geographic locations of the populations under study, and *when* dietary exposure is assessed in relation to cancer outcomes.

In their meta-analysis of 19 prospective studies, Aune *et al* suggest that significant associations between cancer incidence and dietary information may largely depend upon *how* one examines the evidence. Consistent with prior studies,[8] they did not find an overall benefit in their analyses that assumed a linear relationship between fruits and vegetable consumption and colorectal cancer. By contrast, modeling a non-linear relationship using fractional polynomial models yielded a highly significant association. Specifically, most of the risk reduction associated with fruits and vegetables intake was attributable to increasing intake above a “threshold” of 100 grams per day (g/day), with relatively little additional benefit associated with higher levels. As a reference, a typical apple weighs over 200 g. Thus, for the general population, these results suggest that there is little benefit to increasing the consumption of fruits and vegetables beyond the levels associated with eating a reasonably balanced diet. Conversely, these data also suggest that failure to consume a minimum level of fruits and vegetables may be considered a modest risk factor for cancer. These findings are consistent with the results of the Polyp Prevention Trial (PPT), a randomized trial which showed that diets with extremely large amounts of fruits and vegetables (as well as high fiber and low fat) failed to reduce the risk of recurrent adenoma when compared to a usual American diet that included at least 200-400 g/day of fruits and vegetables.[12] Taken together, we can reasonably conclude that increasing consumption of fruits and vegetables does not have a strong anti-cancer benefit for the general population, with any realistic effect limited to individuals with extremely low baseline levels of consumption.

Aune *et al's* results also suggest that *where* – both anatomically and geographically –one looks for an association between fruits and vegetables intake and colorectal cancer matters. They found that higher intake of fruits and vegetables are associated with a modest, yet significant reduction in risk of colon, but not rectal, cancer. Such a biological difference is plausible in light of emerging evidence of distinct risk factors and molecular profiles associated with cancers that arise in the colon compared with the rectum.[13-15] Thus, prior studies with limited ability to examine fruits and vegetables in relation to tumor site may have been unable to detect modest associations. Aune *et al* observed also a significant difference in findings depending on the geographic location of the population under study. Specifically, inverse associations between fruit and vegetable intake were primarily observed in studies of populations from Europe but not the U.S. or Asia. Assuming measurement error does not significantly vary by geography, these results could be explained by several factors. First, fruits and vegetables may exert distinct biological effects according to genetic or environmental factors unique to specific populations. Second, the mean intake of fruits and vegetables in the reference category of “low” intake was markedly lower in the studies from Europe (155 g/day) compared with the U.S. (200 g/day) and Asia (217 g/day). Thus, European studies included a sharper contrast between individuals with “high” intake of fruits and vegetables and individuals with truly “low” levels of intake. By

contrast, in the U.S. and Asian studies, a significant proportion of individuals in the "low" category actually consumed a reasonable amount of fruits and vegetables that might exceed the "threshold" level needed to minimize cancer risk. Lastly, the stronger effect of fruits and vegetables observed by Aune *et al* in the European studies may be in part due to the generally greater contribution of fruits and vegetables to overall folate intake in Europe compared with the U.S., in which there is a higher prevalence of use of folate-containing multivitamins. Folate, an essential micronutrient for DNA synthesis, repair, and methylation, has been promoted as the leading phytochemical candidate responsible for the anti-cancer benefit of fruits and vegetables. Thus, if folate is the primary mediator of the anti-cancer effect of fruits and vegetables, it may be more challenging to detect a small incremental benefit of increasing intake of these foods in a more folate-replete population such as in the U.S..

Although this latter explanation is plausible, the role of folate as a chemopreventive agent has become less certain with the results of randomized controlled trials conducted among individuals with a history of colorectal adenoma. In two trials, folic acid, the synthetic form of folate, did not reduce the risk of recurrent adenomas,[16-18] with a third trial actually observing an increased risk of advanced adenoma and prostate cancer.[18, 19] Taken together with animal studies demonstrating that excess folate during later stages of carcinogenesis could promote carcinogenesis,[20] concerns have been raised that folic acid supplementation or even high levels of dietary folate could actually increase risk of colorectal cancer. The potential mechanism underlying this hypothesis is that additional provision of folate above adequate levels could tip the balance of DNA precursors toward hypermethylation in cancerous cells. In the U.S., this is a considerably worrisome prospect since folic acid fortification in enriched flour began in 1996,[21] temporally associated with a rise in the rates of colorectal cancer in the late 1990s.[22] Recently, this has led to calls to reconsider programs of mandatory folic acid fortification of the food supply despite its established benefit for the prevention of neural tube defects.[23]

In this issue of *Gastroenterology*, a separate study, by Stevens *et al*, helps to allay concerns about a potential pro-carcinogenic role of folate. In a prospective analysis of 99,523 U.S. participants enrolled in the Cancer Prevention Study II (CPS-II) Nutrition Cohort, the investigators examined the association between folate and its various forms (e.g. dietary vs. supplement; natural vs. synthetic) and colorectal cancer incidence from 1999 through 2007, a period entirely within the post-fortification period. Overall, they observed that folate derived from any form -- including folate derived from supplements or folic acid -- was not associated with an increase in colorectal cancer. In fact, total folate was associated with significant 19% lower risk, consistent with studies conducted in the pre-fortification era.[24] These results are remarkably consistent with our recent findings in two large prospective cohort studies which included 87,861 women enrolled in the Nurses' Health Study (NHS) and 47,290 men enrolled in the Health Professionals Follow-up Study (HPFS).[25] In these 2 cohorts, we observed that total folate intake in the post-fortification era was similarly not associated with an increased risk of colorectal cancer.

Notably, the results of Stevens et *al* in evaluating *when* one examines the folate-cancer association provide additional evidence that is central to our understanding of the complex role of this nutrient in the multi-step model of colorectal carcinogenesis. In their analysis, inverse associations between folic acid or total folate and colorectal cancer was significantly influenced by follow-up time, with lower risk limited to cases diagnosed in 2002-2007, 3 to 8 years after assessment of intake. Moreover, inverse associations were particularly strong among those who had ever undergone endoscopy, a population presumably less likely to already have baseline adenomas or early cancer. This suggests that folate may have a weaker or non-existent effect on already established lesions. These findings are consistent with our

results in the NHS and HPFS which demonstrated that folate intake 12-16 years prior to diagnosis was significantly inversely associated with colorectal cancer (relative risk 0.69; 95% CI, 0.51-0.94 for ≥800 compared with <250 μg folate/day), but intake in the more recent past was not. For colorectal adenoma, we observed a strong inverse association with intake 4-8 years prior to diagnosis (odds ratio 0.68; 95% CI, 0.60-0.78 for ≥800 compared with <250 μg folate/day). Based upon current understanding of the prolonged dwell-time of the adenoma before carcinoma develops, our results are consistent with a short-term influence of folate on adenoma initiation. Taken together, the results in CPS II, NHS, and HPFS suggest that folate may inhibit the earliest stages of colorectal carcinogenesis, with a diminished to non-existent influence on progressively more advanced lesions (Figure 1).

The importance of *when* one is exposed to folate demonstrated by these studies also helps explain the discrepant results between epidemiological studies and the null results of the 3 randomized intervention trials of folic acid in adenoma recurrence. First, the intervention studies examined the effect of folic acid in patients with established neoplasia (adenoma). Thus, if folate is indeed effective only in inhibition of the earliest stages of carcinogenesis, the colon of patients with prior adenoma may already have preexisting subclinical neoplasia or a "field defect" that folic acid is unable to reverse.[26] Second, as observed for intake of fruits and vegetables in the control arm of the PPT, the control arm in these intervention trials may have already consumed the minimum level of folate needed to circumvent pro-carcinogenic pathways. In support of this explanation, trial participants with low plasma folate concentrations at baseline that were randomized to folic acid did experience a decrease in adenoma recurrence.[17, 27]

In summary, these two studies apply more rigorous scrutiny to the association between fruits, vegetables, folate, and cancer, highlighting several key points. First, extremely high consumption of fruits and vegetables does not appear to have a substantial anti-cancer benefit. However, diets characterized by extremely low levels of these foods could pose an increase in risk that can be easily minimized with modestly increasing intake. Second, together with our data from 2 other large cohorts,[25] these studies confirm that high doses of folic acid do not appear to increase risk of cancer. This should reassure us that efforts to fortify the food supply to prevent neural tube defects are not causing harm. Third, there remains some evidence that increasing long-term folate intake, particularly among folate-deficient populations, may augment existing colorectal cancer prevention efforts. Fourth, these studies highlight the complexity of diet-cancer relationships and support the importance of accounting for baseline nutritional status, timing of intervention, and examining endpoints that span the spectrum of neoplasia (e.g., initial adenoma vs. recurrent adenoma vs. cancer) and anatomic subtype (e.g., colon vs. rectum). In future studies, as we improve our understanding of the molecular heterogeneity of colorectal cancer, it will be important to examine diet in relation to molecular subtypes of cancer in which specific mechanistic pathways may be more susceptible to dietary intervention.[28, 29] Finally, data supporting a benefit for fruits and vegetables in prevention of cardiovascular disease continue to accumulate.[30, 31] Thus, although increasing consumption of fruits and vegetables may have a relatively minor, if not negligible, impact on colorectal cancer incidence,[2] there remain cogent reasons to continue recommending higher intake for the general population.

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