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The Epidemiologic Evidence and Potential Biological Mechanisms for a Protective Effect of Dietary Fiber on the Risk of Colorectal Cancer

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Abstract Cancers of the colon and rectum represent a significant cause of morbidity and mortality worldwide with the burden especially high in North America, Europe, and in some parts of Asia. More than half of the disease burden has been attributed to an inappropriate diet and lifestyle. Low intakes of dietary fiber are considered to be a risk factor for colorectal cancer, although the epidemiological evidence until now has been conflicting in part due to the difficulties in reliably examining the relationships between components of the diet and disease outcomes due to bias, confounding, and measurement error. Results from recently published, large, prospective, cohort studies and from a meta-analysis of the evidence provide "convincing" evidence of an independent dose-response relationship between total dietary fiber intake and increasing risk of colorectal cancer. The anticarcinogenic properties of fiber on cancers of the colon and rectum, however, have still to be elucidated.

Keywords Epidemiology · Dietary fiber · Colorectal cancer · Biological mechanisms

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Introduction

Combined, cancers of the colon and rectum (colorectal) constitute approximately 10 % of all cancers worldwide, and more than half a million people die from colorectal cancer annually—equivalent to approximately 8 % of all cancer-related deaths worldwide [1]. The burden of this disease is particularly high in developed countries where these malignancies rank second in terms of both incidence and mortality compared with approximately fifth in lower- and middle-income countries [1]. The occurrence of colorectal cancer varies at least 25-fold between countries [1, 2] with the highest incidence rates for colorectal cancer seen in certain areas and ethnic groups in the United States, Canada, Japan, and New Zealand and the lowest rates in South East Asia and Africa [3]. It is this wide geographical variation in incidence rates for colorectal cancer, combined with data from migrant studies [4] that strongly suggest that modifiable risk factors, including diet [5•, 6, 7], physical activity [5•, 8], obesity [5•, 9], cigarette smoking, and diabetes [5•, 10], play a pivotal role in the aetiology of this malignancy [5•, 6–11] (Fig. 1). Indeed, an inappropriate diet, combined with physical inactivity, has been estimated to explain more than half of the disease burden for colorectal cancer [1].

With respect to diet, the components that are considered to have a causative role in the disease, and for which there is the strongest evidence, include high intakes of red and processed meat and high alcohol consumption, whereas intake of dietary fiber is suggested to have a protective effect [5•, 12]. However, the magnitude of these associations is generally weak (Fig. 1), which often has hindered their detection in epidemiological studies where confounding, measurement error, and other forms of bias can drown out

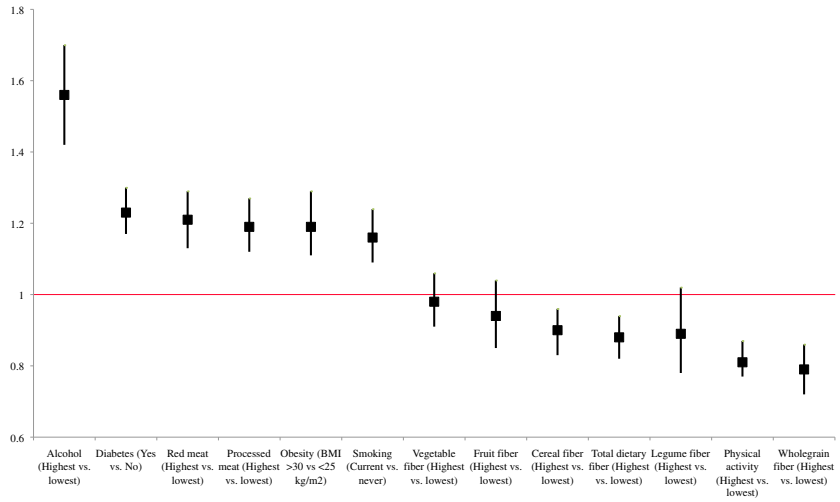

Fig. 1 Relationships between dietary and lifestyle risk factors and risk of incident colorectal cancer as reported in two published meta-analyses from Huxley et al. [5•] and Aune et al. [24••]. (Black square) pooled estimates of effect size; (vertical line) 95 % confidence intervals for the observed effect in the pooled estimate. The horizontal line represents unity. The comparison groups are shown for each of the diet and lifestyle variables assessed; for alcohol, physical activity, red and processed meat, and for all sources of fiber intake the comparison was for the highest compared with the lowest intake. BMI, body mass index

any underlying signal. Therefore, the relationships between an exposure of interest and disease outcome often only become apparent in very large, well-conducted cohort studies (such as the European Prospective Investigation into Cancer and Nutrition [EPIC]) [13] or meta-analyses of cohort studies. However, even then there may still be insufficient statistical power to observe a significant association (e.g., in the Pooling Project [14]) if the signal to noise ratio is low.

Moreover, examining the relationship between dietary fiber and risk of colorectal cancer in epidemiological studies is beset by several other important limitations. First, dietary fiber is a term that comprises a wide variety of plant matter, including non-starch polysaccharide (NSP), resistant starch, inulin, lignin, waxes, chitins, pectins, beta-glucans, and oligosaccharides—not all of which are measured in cohort studies and which may each have differing effects on the risk of disease [15]. Second, individuals who have diets that are high in fiber also tend to have other healthy behaviors, such as consuming relatively low amounts of saturated fat, red and processed meat, and alcohol. Therefore, in epidemiological studies it often is very difficult, if not impossible, to completely disentangle the effect of diets that are rich in fiber from other healthy diet and lifestyle behaviors, resulting in residual confounding.

The first part of this review describes the most recent epidemiological advances pertaining to the role of dietary fiber on subsequent risk of cancers of the colon and rectum, which for the purposes of this review are considered collectively as colorectal cancer. The second part details recent advances in understanding possible biological mechanisms by which dietary fiber impacts the risk of developing this malignancy.

Recent Advances in the Epidemiological Evidence

In 2007, the World Cancer Research Fund (WCRF) [12] released the Second Expert Report summarizing the evidence for the role of diet and lifestyle risk factors on risk of cancers of the colon and rectum. This report concluded that there was “probable” evidence for a protective effect of foods containing dietary fiber on risk of colorectal cancer, based largely on a pooled analysis of 13 prospective cohort studies with information from more than 725,000 individuals and 8,000 cases of colorectal cancer from North America and Europe [14]. In that analysis, there was a significant inverse association between fiber intake and risk of colorectal cancer in the age-adjusted model (pooled relative risk (RR)=0.84; 95 % confidence interval (CI), 0.77–0.92), but once consideration was made for relevant confounders, including other dietary components, body mass index (BMI), smoking, and sociodemographic characteristics, the association was attenuated and no longer statistically significant: pooled RR=0.94; 95 % CI, 0.86–1.03.

Since the WCRF report [12], several other, large, prospective, cohort studies have been published that evaluated the relationship between dietary fiber and risk of colorectal cancer [16–23]. In 2011, all of the available data from published cohort studies that had reported on the relationship between dietary fiber and colorectal cancer were combined in a meta-analysis [24••]. This overview included data from 25 prospective cohort studies from the United States, Europe, and Asia that were conducted within the past 25 years, with information on nearly two million individuals and more than 14,000 cases of colorectal cancer, making it the largest dataset on this topic. In that review, Aune and

colleagues reported that of the 19 studies (most of which had adjusted for relevant confounders) reporting on the relationship by high versus low total dietary fiber intake, those individuals with the highest intakes had a statistically significant 12 % (95 % CI, 6–18) reduction in the risk of colorectal cancer compared with those with the lowest intakes. Furthermore, there was no evidence of important heterogeneity between studies (i.e., estimate of effect was broadly consistent across different studies). A total of 16 studies with information on nearly two million individuals and more than 14,000 cases of the neoplasm contributed to a dose-response analysis, which showed that for every 10 g/day of total dietary fiber intake, the risk of colorectal cancer significantly decreased by 10 % (95 % CI, 6–14) [24••].

A range of sensitivity analyses was conducted in an attempt to ascertain whether the positive association with colorectal cancer was present for all sources of dietary fiber. The authors examined the relationship between fruit, vegetables, legumes, cereal, and whole grains and reported that there was only statistically significant evidence of a protective association for fiber derived from cereals (pooled RR per 10 g of cereal fiber: 0.9; 95 % CI, 0.83–0.97) and from an extra three servings daily of whole grains (90 g) (pooled RR, 0.83; 95 % CI, 0.78–0.89). Because some of these analyses were based on data from only four studies, the lack of a significant association with some sources of dietary fiber may be due more to measurement error and associated noise than any true lack of a relationship [24••].

Shortly after the updated meta-analysis by Aune and colleagues was published [24••], updated findings of the association between fiber intake and risk of cancers of the colon and rectum from EPIC—a large multicenter cohort study of more than half a million individuals—appeared. These were based on a much longer duration of follow-up than in the previous EPIC publication that was included in the review by Aune and colleagues (11 yr vs. 6 yr) and thus many more incident cases (4,517 vs. 1,721) [25•]. Importantly, 23 of the EPIC study centers used the same standardized methodology to examine relationships between exposures and outcomes and hence they were able to correct partially for the effect of dietary assessment measurement error through regression calibration. The key findings from this study were in line with those of the meta-analysis by Aune and colleagues [24••] and provided more evidence of a modest and independent dose-response relationship between dietary fiber and risk of colorectal cancer; in the model that was adjusted for a range of risk factors and confounders, including total energy intake, BMI, physical activity, and alcohol and meat intake, each 10 g/day increase in fiber was associated with a significant 13 % (95 % CI, 4–21) reduction in the risk of colorectal cancer. Moreover, for colon cancer the protective effect of dietary fiber was apparent for all sources of fiber (cereals, fruits, and vegetables), whereas for rectal cancer, only fiber from cereals was significantly associated with reduced risk.

In 2011, the WCRF's Second Expert Report [12] was updated and revised to account for the accumulating evidence for a protective effect of dietary fiber on risk of colorectal cancer. From there being only "possible" evidence of an association in 2007, the association was now considered to be "convincing," although the group noted that evidence gaps still remain pertaining to the impact of fiber intake on colorectal subsites and the relationship between sources of dietary fiber with cancer risk [26••]. Although not directly comparable, the size of the inverse association between total dietary fiber intake (particularly from wholegrains) and risk of developing colorectal cancer is similar to that for physical activity—the only other modifiable risk factor that is considered to be protective against this malignancy (Fig. 1).

Potential Biological Mechanisms for Fiber Protection against Colonic Cancer

Numerous biological mechanisms have been suggested to explain the anticarcinogenic properties of fiber, including decreased bowel transit times, dilution of fecal carcinogens, increased phenolic acids, lowering of fecal pH (which leads to lower absorption of carcinogens), increased butyrate production, and decreased ammonia, phenols, and indoles [27]. Many of these observations were made many years ago, and only the most recent observations will be reviewed in detail here. The most active area of research is concerned with defining the microbiota (i.e., the bacterial populations living in the human gut) in normal and diseased states and the effect of diet (including fiber) on the microbiota. In recent years, there has been accumulating interest about the possible role that the gut microbiome may have in the etiology of certain diseases, such as irritable bowel disease, Crohn's disease, diabetes, and even obesity [28]. We concentrate on the evidence regarding a possible relationship between the microbiome and carcionogenesis.

Impact of Diet on the Microbiome

Human feces are genotoxic and cytotoxic to colon cells [29, 30]. Studies in germ-free mice have shown that fewer cancers are formed with chemical carcinogens compared with mice with normal faecal bacteria [31]. Bacteria are capable of forming genotoxic products from normal dietary components, especially meat protein [32]. Information regarding the species and functional composition of the human gut microbiome is limited to data from a handful of cohorts with little evidence regarding variation across the world. Arumugam and colleagues performed the equivalent of a meta-analysis by combining 22 newly sequenced fecal

metagenomes of individuals from four countries with previously published data sets. They identified three robust clusters of gut bacteria (referred to as enterotypes) that were not nation- or continent-specific [33]. These enterotypes were identified by the variation at the level of one of the three following genera: *Bacteroides*, *Prevotella*, and *Ruminococcus*. These enterotypes were not correlated with the BMI, age, gender, or nationality of the subjects studied. However, definition of the microbial species more often present in people who develop adenomas and carcinomas (other than those specifically on the adenoma or carcinoma), compared with normal healthy people, has not yet been done. The Human Microbiome Project [34•, 35] has defined the normal fecal flora in more than 200 healthy men and women younger than aged 45 years. In that study, the major genera using the relatively new technique of 16S Ribosomal RNA typing was shown to be *Bacteroides* followed by *Prevotella* (both gram-negative bacteria). In those individuals with low *Bacteroides*, *Fermicutes* (gram-positive bacteria) was the major phylum present.

In a subsequent study published in *Science*, Wu and colleagues performed investigations into the relationship between diet and prevalence of enterotype using healthy volunteers [36••]. Using food-frequency questionnaires and obtaining stool samples from the 98 volunteers from which they could analyze DNA content to provide a snapshot of the gut microbiota, the authors showed that the *Bacteroides* enterotype was more common among individuals who reported eating a lot of meat and saturated fat, whereas in those who reported eating diets that were rich in carbohydrate, *Prevotella* was the dominant enterotype. In individuals consuming alcohol and high intakes of polyunsaturated fat, *Ruminococcus* was the most common enterotype. Wu and colleagues then performed a controlled-feeding study of ten subjects and showed that the composition of the microbiome changed within 24 hours after initiating a high-fat/low-fiber or low-fat/high-fiber diet, but that the enterotype identity remained stable during the 10-day study [36••]. These data suggest that the composition of the microbiome is influenced by diet and that it may be feasible to alter the enterotype through long-term dietary manipulation.

Obesity and the Microbiome and Carcinogenesis

Obesity is clearly linked to colorectal cancer [5•], and this link may be via the postulated differences in *Bacteroides* and *Firmicutes* between lean and obese subjects [35]. However, other investigators have found no differences in the microbiome between lean and obese subjects [37], and no changes with weight loss. It is possible that obesity could be linked to colorectal cancer via lower levels of *bifidobacteria*, which has been noted in obese women (who have higher levels of *Bacteroides*, *Clostridium*, and *Staphylococcus*) who gain excessive weight during pregnancy and also in people with type 2 diabetes—itself a putative risk factor for colorectal cancer [5•, 38, 39]. Weight loss also has been associated with reduced level of *Bifidobacterium bifidum* and *Bifidobacterium breve* counts and increased *Bifidobacterium catenulatum* [40]. The level of *Bifidobacterium* genus also is decreased upon weight loss after bariatric surgery [41].

Although there is a large amount of mouse data, there are little human data on the possible interactions between obesity, the microbiome, and the response to different macronutrients, including fiber. It has been shown that an increase in calorie intake (from 2,400 to 3,400 kcal/d) in obese and lean human individuals promotes rapid changes in the gut microbiota (20 % increase in *Firmicutes* and a corresponding decrease in *Bacteroidetes*) [42]. This was associated with an increased energy harvest of ≈150 kcal; the overfeeding in lean individuals was accompanied by a greater fractional decrease in stool energy loss [42]. The controversial research pertaining to the relationship between obesity and the microbiome has been extensively reviewed by Delzenne [43].

Effects of Nonabsorbable Carbohydrates on the Microbiota

Prebiotics are nondigested carbohydrates, which alter gut bacteria amounts, types, and activities in a way that is presumed (but not proven) to be favorable. In general, after prebiotics, *bifidobacteria* and *lactobacilli* increase and *clostridia* and *coliforms* decrease, but whether these bacteria are related to colorectal cancer is not known [44]. Weickert and colleagues [45] contrasted 43 g of fiber with 14 g of fiber (mostly cellulose and hemicellulose, and thus poorly fermentable) over 18 weeks in 69 people and measured butyrate producing species: no changes in species were seen with fiber, suggesting that at least for nonfermentable fiber, its potential protection from colorectal cancer is not via species change.

Walker and colleagues [46] performed a controlled feeding study in six overweight men with resistant starch (RS), nonstarch polysaccharide (NSP) and a reduced carbohydrate weight loss diet over 10 weeks to examine stool 16s RNA sequences; 320 phylotypes were identified. Changes in bacterial groups occurred with diet but there was marked individual variation and samples clustered more by individual than by diet. Relatives of *Ruminococcus bromii* increased in most volunteers on the RS diet, accounting for a mean of 17 % of total bacteria compared with 3.8 % on the NSP diet, whereas the *Oscillibacter* group increased on the RS and weight loss diets. Relatives of *Eubacterium rectale* increased on RS (to mean 10.1 %) but decreased on the weight loss diet. Interindividual variation was marked, however, with >60 % of RS remaining unfermented in two volunteers on the RS diet, compared with <4 % in the other 12

volunteers; these two individuals also showed low numbers (<1 %) of *Ruminococci*.

Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and lactulose are readily fermented and selectively stimulate particular species of bacteria usually *bifido* bacteria [44]. Consumption of 5–10 g of GOS led to five- to tenfold increases in *bifidobacteria* in half of the subjects with decreases in *Bacteroidetes*. Increases in *Firmicutes* also were observed in a few individuals. The responses to GOS and the magnitude of the response varied between individuals were reversible and were dose-related [47]. GOS enriches different lineages within the genus *Bifidobacterium* compared with resistant starches [48]. RS4 (chemically modified starch) but not RS2 (resistant granules) significantly increased *Actinobacteria* and *Bacteroidetes* while decreasing *Firmicutes*. RS4 increased *Bifidobacterium adolescentis* (in some subjects up to 18–30 % of the population) and *Parabacteroides distasonis*, whereas RS2 increased *Ruminococcus bromii* and *Eubacterium rectale* compared with RS4. The responses to resistant starch and the size of the change varied between individuals. The changes were reversible and associated with the consumption of RS.

The Butyrate Hypothesis and Risk of Colorectal Carcinogenesis

The effects of butyrate on slowing the growth of colon cancer cells lines was first described in 1980 [49]. There have been more than 600 publications on butyrate since then, mostly in experimental animals and *in vitro* systems. In humans, there is no known evidence that butyrate levels or production and fecal pH is different in those with and without colorectal cancer [50, 51]. A recent review examined the potential mechanisms of butyrate protection from colorectal carcinogenesis [52]. Butyrate slows cancer cell proliferation and induces differentiation and apoptosis, via inhibition of histone deactylase, but also inactivates carcinogens, through glutathione-S transferase enzyme induction. The mechanism of the GST induction may be via histone deactylase inhibition and modulation of MAPK pathways through ERK phosphorylation. Butyrate induces the phase 2 enzyme Glutathione S-transferase and NAD(P)H:quinone oxidoreductase (NQO) in a dose-dependent manner in rat intestinal cells. This was related to an increase of NF-E2-related factor 2 (Nrf2) nuclear translocation and a decrease in the levels of nuclear fraction p53 mRNA [53].

Butyrate signals via gut G protein coupled receptors GPR41, GPR43, and GPR109A the expression of which is frequently lost in colonic cancer cells [54]. Apoptosis induced by activation of GPR109A with butyrate in colon cancer cells does not involve inhibition of histone deacetylation. The primary changes in this apoptotic process include down-regulation of Bcl-2, Bcl-xL, and cyclin D1 and up-regulation of death receptor pathways. In addition, GPR109A/butyrate suppresses nuclear factor-kappaB activation in normal and cancer colon cell lines as well as in normal mouse colon. GPR109A is silenced in colon cancer cells [55].

Germ-free animals have low levels of GPR109A, a butyrate receptor, and SLC5A8, a butyrate transporter in the ileum and the colon. The expression returns to normal levels when germ-free mice are colonized with bacteria. Microarray analysis identifies approximately 700 gut genes whose expression is altered more than twofold in germ-free mice compared with conventional mice. Among these genes are the chloride/bicarbonate exchanger SLC26A3 and the water channel aquaporin 4 [56].

Increasing butyrate by attaching it to resistant starch in the colon of AOM-treated rats increases apoptosis by 50–70 % 6 hours after AOM [57], resulting in lower large bowel tumor numbers after 25 weeks [58]. Resistant starch without added butyrate lowered tumor numbers but did not significantly change apoptosis rates, possibly suggesting different mechanisms of protection. Rates of apoptosis were not increased by red meat or increased amounts of protein but were lower with resistant starch (presumably reflecting less DNA damage) and protein fermentation products (fecal ammonia and phenol) also were reduced in mice fed resistant starch reflecting diversion of these components into microbial synthesis stimulated by carbohydrate [59].

Red meat in mice increases the promutagenic adduct O(6)-methyl-2-deoxyguanosine in colonocytes compared with casein, although there was no dose effect on either diet, whereas mice fed 10 % high amylose starch had reduced levels of the adduct and increased short chain fatty acids. These results differ from the results seen with the comet assay in rats where increasing casein and soy from 15 % to 25 % increases DNA damage, which is partially reversed by a very high resistant starch diet (48 % high amylose starch) but the effects on DNA damage were *unrelated* to fecal short chain fatty acids [60]. High amylose starch with butyrate attached increased fecal butyrate and was twice as effective as normal high amylose starch at reversing high protein-induced DNA damage. High protein diets on a background of low-amylose wheat or a novel high amylose wheat diet did not increase DNA damage [61].

Impact of Weight Loss

Weight loss diets that have reduced carbohydrate and increased protein are speculated potentially to increase the risk of colorectal cancer (despite the likely protective effect conferred by weight loss itself) by lowering butyrate concentrations [62] and lowering the number of butyrate producing bacteria [63] and increasing the proportions of branched-chain fatty acids and concentrations of phenylacetic acid and

N-nitroso compounds. Very low carbohydrate diets (22 g/d) in 17 obese men for 4 weeks reduced the *Roseburia/Eubacterium rectale* group of bacteria (*Firmicutes* phylum, *Lachnospiracea* family) and greatly reduced concentrations of fiber-derived, antioxidant phenolic acids, such as ferulate and its derivatives [64] compared with high carbohydrate diets (360 g/d). There was no significant change in the overall proportion of *Lachnospiraceae* or in a second major group of butyrate-producing gram-positive bacteria related to *Faecalibacterium prausnitzii*. The proportion of *Bacteroides* spp. decreased by 22 % with the very low carbohydrate diet compared with that with high carbohydrate diets and the total number of bacteria was greater ($P<0.012$) with the high carbohydrate diet than with the other two diets with lower amounts of carbohydrate.

In summary, the proposition that differences in microbial population underlie population differences in colorectal cancer was extensively investigated in the 1970s, without a clear outcome because of problems with culturing many species. This question is now being addressed with molecular techniques; at present the only data show differences in the microbiome adherent to adenomas [65] and carcinomas [66, 67], although this may have nothing to do with causation and may merely reflect changes in mucin layers in the tumors. It is not clear if luminal or mucosal bacteria (or both) are the important players in carcinogenesis. Nor is it clear that bacteria in the *Bacteroidetes* phylum are beneficial and those in the *Fermicutes* phylum are harmful, as butyrate-producing species are found in both, whereas *bifidobacteria* may be helpful and *lactobacilli* may be harmful. Weight loss appears to reduce some butyrate-producing species. Moreover, the response of the microbiota to different forms of fiber are very variable and, if protection from colorectal cancer is related to shifts in microbial populations with fiber, then only a small proportion of the population on a high fermentable fiber diet will gain protection, thus diluting the overall effect seen in a general population study.

Conclusions

In 2011, in the wake of findings from several, large, prospective, epidemiological studies, the WCRF revised their consensus statement of a "probable" protective effect of dietary fiber on subsequent risk of developing colorectal cancer. This scientific body now considers the inverse association between fiber intake and risk of colorectal cancer to be supported by a "convincing" level of evidence. The magnitude of the association is comparable to that reported for physical activity, the only other modifiable lifestyle for which there is strong epidemiological evidence of a protective effect against developing this malignancy. However, uncertainty remains regarding the specific impact of dietary fiber on subsites of colorectal cancer and whether the protective effect of fiber on subsequent risk of the malignancy is apparent irrespective of its dietary source. Moreover, the biological mechanisms by which dietary fiber exerts its anticarcinogenic effects have yet to be fully elucidated, although there have been some recent exciting developments relating to the role of the microbiome. Nevertheless, the data are sufficiently robust to warrant the incorporation of the recommendation for increasing total dietary fiber intake alongside those advocating increased physical activity public health guidelines for the primary prevention of this highly preventable malignancy.

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- • Of importance
- •• Of major importance

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