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Diet and cancer prevention

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Abstract

Research from several sources provides strong evidence that vegetables, fruits, and whole grains, dietary fibre, certain micronutrients, some fatty acids and physical activity protect against some cancers. In contrast, other factors, such as obesity, alcohol, some fatty acids and food preparation methods may increase risks. Unravelling the multitude of plausible mechanisms for the effects of dietary factors on cancer risk will likely necessitate that nutrition research moves beyond traditional epidemiological and metabolic studies. Nutritional sciences must build on recent advances in molecular biology and genetics to move the discipline from being largely 'observational' to focusing on 'cause and effect'. Such basic research is fundamental to cancer prevention strategies that incorporate effective dietary interventions for target populations.

Keywords: Cancer prevention; Diet and cancer; Folate; Gene–nutrient interactions; Genetic polymorphisms

1. Introduction

Diet plays a major role in cancer aetiology and prevention. Although inconsistencies exist across studies that have investigated the relationship between diet and cancer, the basic assertion that dietary factors influence cancer risk is not really a matter of debate. Nevertheless, many questions remain to be resolved, including exactly which specific dietary factors are most closely linked to cancer prevention, by what mechanisms food components exert their putative effects, how dietary factors might interact to affect cancer risk and what preventive steps can be taken to minimise adverse effects of factors that appear to increase disease risk. The complexity of cancer means these questions will not have simple answers. For example, a genome-wide search for deleted regions in 75 human primary breast tumours identified 56 different regions of the genome with loss of heterozygosity (LOH); the remarkable finding was that all tumours had different sets of deletions [1]. Such heterogeneity, reflecting various genetic alterations and pathways to disease, has a major impact on efforts to establish linkages between diet and cancer, no matter how rigorously studies are designed and performed [2]. Likewise, interindividual variations in susceptibility arising from common polymorphisms in genes governing the metabolism of exogenous substances can modify the carcinogenic or anticarcinogenic effects of food components and, thus, add an extra level of difficulty to the interpretation of studies [3,4]. This paper briefly summarises evidence for a diet-and-cancer relationship, noting its relevance for prevention; discusses plausible mechanisms-of-action; highlights the role of gene–nutrient interactions in determining cancer risk; and considers the benefits of a molecular-based approach in the design and conduct of future research studies aimed at elucidating the diet-and-cancer relationship and, ultimately, at developing optimally effective cancer prevention strategies.

2. Evidence for a diet and cancer relationship

Epidemiological studies, supported by preclinical data from animal and *in vitro* experiments and by clinical findings, have contributed immensely in providing insights into links between diet and cancer prevention

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and to the development of diet and cancer hypotheses for testing in clinical trials. Although a powerful research method, the value of epidemiology in establishing relationships between diet and cancer is not without limitations — for example, measurement errors in dietary assessment and interactions of dietary components that lead to confounding of the study results. Nevertheless, certain epidemiological data linking dietary factors and cancer are remarkably consistent; for example, evidence consistently supports an inverse relationship between cancer risk and intake of vegetables and fruits [5,6]. Overall, the body of current available data supports inverse relationships between cancer risk and intakes of vegetables, fruits, whole grains, dietary fibre, certain micronutrients and certain types of fat (e.g. n-3 fatty acids, particularly n-3/n-6 ratios), as well as physical activity; and direct relationships between cancer risk and intakes of total fat/certain types of fat (e.g. saturated fat) and alcohol, as well as obesity (as measured by a high body mass index (BMI)) and certain food preparation methods such as smoking, salting and pickling foods, and high-temperature cooking of meats [5–12].

Evidence-based dietary guidelines likely to reduce cancer risk have been formulated by various organisations [5,13,14] and generally propose that individuals should reduce fat intake, particularly from animal sources, increase fibre intake, include a variety of vegetables and fruits in the daily diet, be physically active and maintain a healthy weight, consume alcoholic beverages in moderation, if at all, and minimise consumption of salt-cured, salt-pickled or smoked foods. A recent analysis of data from the Health Professionals Follow-Up Study (HPFS) linking modifiable risk factors (red meat consumption, obesity, low folic acid intake, physical inactivity, alcohol consumption and early adulthood cigarette smoking) to colon cancer risk found that 39, 48 and 55% of colon cancer risk in this cohort of middle-aged American men might be avoidable if all men were in the bottom 20, 10 and 5% of the risk scores, respectively [15].

Ongoing large epidemiological studies, such as the European Prospective Investigation into Cancer and Nutrition (EPIC), have the potential to provide additional valuable insights into the roles that specific dietary factors have in cancer aetiology. EPIC, a prospective study with approximately 460 000 subjects, is being carried out in 22 centres across nine European countries. It is designed to investigate the relationship between diet, nutritional and metabolic characteristics, various lifestyle characteristics and cancer risk and includes collection and long-term storage of blood samples to be used in measuring potential cancer biomarkers [16]. Studies that include a molecular epidemiology component, such as EPIC, can help to assess variations in risk across populations more accurately and to identify particularly cancer-susceptible subgroups within populations, thus facilitating the development of effective approaches to cancer prevention [17].

2.1. Vegetables and fruits

Epidemiological data overwhelmingly support the apparent inverse association between consumption of vegetables and fruits and cancer risk, as demonstrated in one comprehensive review of more than 250 case–control and cohort studies. These studies, conducted in countries with diverse dietary practices, evaluated risks for various types of cancer using a number of different dietary assessment techniques [6]. Despite limitations in methodology and variations across studies, convincing evidence for inverse risk associations with vegetable and fruit intake exists for cancers of the mouth and pharynx, oesophagus, lung, stomach, colon and rectum [5,6]. Recent reports from the Netherlands Cohort Study [18] and a European multicentre study [19] confirm inverse associations for both vegetable and fruit intake and lung cancer; the strongest effects were observed for *Brassica* vegetables (relative risk (RR) = 0.5; 95% CI = 0.3–0.9) [18] and tomatoes (odds ratio (OR) = 0.5; 95% CI = 0.4–0.6) [19]. Unexpectedly, one large prospective study recently reported that frequent consumption of vegetables and fruits did not affect the risk for colon and rectal cancers [20]. Data linking vegetable and fruit intake to breast cancer risk are not consistent, overall [5,6]. One meta-analysis of 26 studies on vegetable and fruit consumption (high versus low) and breast cancer risk indicated a greater reduced risk for vegetables (RR = 0.75; 95% CI = 0.66–0.85) than for fruits (RR = 0.94; 95% CI = 0.79–1.11) [21]. The most consistent associations appear to be inverse associations for consumption of green vegetables and carrots [6]. Overall, total vegetable and fruit consumption does not appear to be associated with prostate cancer risk [6]. One recent study, however, reported significantly reduced risk of prostate cancer for both total (RR = 0.65; 95% CI = 0.45–0.94) and cruciferous (RR = 0.59; 95% CI = 0.39–0.90) vegetable consumption [22]. Some evidence suggests a benefit for prostate cancer from yellow vegetables and tomatoes [6].

Data from the EPIC-Italy study show strong negative associations between levels of leucocyte DNA adducts, a biological marker that might be predictive of cancer risk [23], and consumption of fresh vegetables and fruits, particularly green leafy vegetables [24]. In addition, evidence exists that increased vegetable and fruit consumption can reduce oxidative damage to DNA [25,26]. Some evidence suggests that vegetable consumption can influence the activity of xenobiotic-metabolising enzymes. For instance, *Brassica* vegetables contain glucosinolates that are hydrolysed to indoles and isothiocyanates, compounds known to have effects

on these enzymes [27]. In clinical studies, *Brassica* vegetables increased activities of cytochrome P450 (CYP) 1A2 [28] as well as glutathione *S*-transferase (GST)-α and GST-μ [29], apiaceous vegetables (e.g. celery, carrots) decreased CYP1A2 activity [28], and *Allium* vegetables (e.g. garlic, onion) increased GST-μ activity [29], illustrating the difficulty of trying to sort out the effects of specific vegetables in observational studies. Consumption of *Brassica* vegetables is also associated with an increased ratio of 2-hydroxyoestrone (not a breast tumour promoter) to 16α-hydroxyoestrone (a breast tumour promoter), which may be a marker for reduced breast cancer risk [30].

In reality, numerous constituents found in vegetables and fruits — including dietary fibre, micronutrients, and various phytochemicals — and interactions among these constituents might contribute to the ability of these foods to reduce cancer risk. Determining which constituents are most effective and how they exert their effects pose significant challenges for the cancer research community.

2.2. Dietary fibre

Evidence from epidemiology suggests that colorectal and breast cancer risk may be decreased by increasing the intake of dietary fibre and fibre-rich foods, including vegetables, fruits, cereals and whole grains [5,10,31,32], but findings are not entirely consistent. For instance, based on 25 years of follow-up data for men in the Seven Countries Study, an increase in fibre intake of 10 g/day was associated with a 33% lower colorectal cancer mortality risk [33]. However, 16 years of follow-up data from the Nurses Health Study (NHS) showed no association between dietary fibre intake and colorectal cancer risk in women [34]. For breast cancer, one recent review found that nine of 10 case–control studies showed an inverse correlation between either cereal consumption or fibre intake, whereas four prospective cohort studies reported either no change in breast cancer risk or a non-significant decrease in risk [35]. Fibres from different sources have been studied for their effects on cancer risk [5,10]. Wheat bran, rich in dietary fibre as well as various phytochemicals and vitamins, is associated with a reduced risk for colon and breast cancers [36,37]. A recent randomised trial, however, reported that dietary supplementation with wheat-bran fibre did not decrease the recurrence of adenomatous polyps, considered to be precursors of most colorectal cancers [38]. Overall, evaluation of the dietary fibre–cancer relationship is complicated by the varying composition of fibre from different sources, variations in fibre measurement techniques and dietary assessment and the possible effects of micronutrients and phytochemicals present in high-fibre foods. For example, recent experimental data demonstrate that the lipid fraction of wheat bran, which contains substances such as tocopherols and phenolic compounds, inhibits colon cancer development [39]. Results from the Polyp Prevention Trial — which investigated whether a diet low in fat (20% of calories), high in fibre (18 g fibre/1000 calories), and high in vegetable and fruit intake (5–8 servings/day) will decrease the recurrence of adenomatous polyps — showed no effect of this diet on polyp incidence [40], similar to earlier findings in the Australian Polyp Prevention Project [41]. Findings from adenoma trials, however, do not provide information about possible effects on later stages of colorectal cancer development [42].

Dietary fibre may influence colon risk through various proposed mechanisms, which include increasing faecal bulk (diluting carcinogens); increasing transit time through the colon (reducing interactions of carcinogens with mucosal cells); direct binding of carcinogens; modifying the mix and the enzyme activities of intestinal bacterial flora (decreasing concentrations of secondary bile acids); and producing short-chain fatty acids (SCFAs) by fermentation, which may inhibit carcinogenesis through effects on colonic pH and increased availability of butyrate [10,43,44]. Butyrate promotes growth arrest (by inducing cyclin-dependent kinase inhibitors, e.g. $p21^{WAF1/Cip1}$), differentiation and apoptosis in colon tumour cell lines [44,45] and in breast cancer cell lines [46]. In one recent study, dietary butyrate inhibited chemically-induced mammary cancer in rats [46]. Several mechanisms have been proposed for butyrate's ability to induce apoptosis, including the stimulation of histone acetylation, which enhances the production of $p21^{WAF1/Cip1}$, and downregulation of bcl-2, an oncogene that acts by blocking apoptotic cell death [44,47,48]. Evidence suggests that fat and fibre may interact to influence apoptosis. Dietary pectin, a fibre that produces high amounts of butyrate during fermentation, enhances the upregulation of apoptosis by fish oil in experimentally-induced colon cancer [47].

2.3. Micronutrients

Commonly consumed foods, particularly vegetables and fruits, are sources of numerous micronutrients. Several of these, including β-carotene (a vitamin A precursor), vitamin E, vitamin C and selenium — which all have antioxidant potential — as well as calcium, vitamin D (in fish, eggs and fortified dairy products), and folate, have been the focus of extensive experimental and epidemiological research to determine their influence on cancer risk [5,49–53]. Reviews of epidemiological studies that correlated either high intakes of β-carotene-rich vegetables and fruits or high blood concentrations of β-carotene with cancer risk have consistently found evidence of a significant inverse association with lung cancer risk [5,54–56]. The epidemiological data linking high intakes of β-carotene-

rich vegetables and fruits to reduced lung cancer risk, along with animal data demonstrating that β-carotene inhibited cancer-related events, provided strong support for testing the effect of β-carotene supplements on lung cancer in clinical interventions in high-risk populations [57,58] and in the general population [59,60]. Large-scale interventions using β-carotene are presented in the clinical trials section. Unexpectedly, results from large-scale trials indicated that β-carotene supplementation may increase lung cancer risk in high-risk individuals [57,58]. Data from general population trials showed no significant evidence of either benefit or harm from β-carotene [59,60]. At present, there is no clear evidence that β-carotene, at dietary levels, reduces cancer risk [49]. It is possible that β-carotene is simply a marker for the actual substances in vegetables and fruits that may inhibit cancer development.

One review of epidemiological data concluded that vitamin E possibly decreases risk for lung and cervical cancers [5]. In a recent large cohort study, however, high vitamin E intake did not reduce lung cancer risk in men [61]. Although supplemental vitamin E was not generally associated with prostate cancer risk in the Health Professionals Follow-up Study (HPFS), the data indicated an inverse association (RR = 0.44; 95% CI = 0.18–1.07) between supplemental vitamin E and risk of metastatic or fatal prostate cancer among current smokers [62]. These findings are supported by a large-scale clinical intervention, in which 34% fewer cases of prostate cancer and 16% fewer cases of colorectal cancer (both secondary endpoints) were diagnosed among male cigarette smokers who received daily vitamin E supplements [57]. Vitamin E succinate (VES), a derivative of vitamin E, has been shown to trigger apoptosis of human prostate carcinoma cells *in vitro* [63].

Epidemiological studies of diets high in vitamin C-containing vegetables and fruits indicate that vitamin C probably decreases risk for stomach cancer and possibly decreases risk for cancers of the mouth, pharynx, oesophagus, lung, pancreas and cervix [5]. Recent case–control data indicated a risk reduction of 40–60% for gastric cancer [64] and 66% for oral/pharyngeal cancer [65] and cohort data indicated a risk reduction of 23% in lung cancer in men [61] for highest versus lowest levels of intake of vitamin C.

Data from most case–control and cohort studies investigating selenium and cancer show a possible inverse relationship with lung cancer [5,54], but data have not been convincing overall for other cancer sites [5,12]. One study of selenium levels in toenails reported a reduced risk of advanced prostate cancer (OR = 0.35; 95% CI = 0.16–0.78) at the highest quintile [66]. Similarly, more recent cohort data indicated overall reduced risk (RR = 0.5; 95% CI = 0.3–0.9) for prostate cancer at the highest quartile of blood selenium levels, but greater reduced risk (RR = 0.3; 95% CI = 0.1–0.8) for advanced disease [67]. Secondary endpoint analyses in a clinical intervention trial to determine whether selenium supplementation protects against the development of non-melanoma skin cancer in skin cancer patients showed no beneficial effect on skin cancer, but did show significant reductions in total cancer mortality (RR = 0.5; 95% CI = 0.31–0.80), total cancer incidence (RR = 0.63; 95% CI = 0.47–0.85) and in incidence of lung (RR = 0.54; 95% CI = 0.30–0.98), colorectal (RR = 0.42; 95% CI = 0.18–0.95), and prostate (RR = 0.37; 95% CI = 0.18–0.71) cancers for individuals who received selenium supplements [68]. Furthermore, recent data from the Linxian General Population Trial indicated a significant association between serum selenium levels and a reduced risk of oesophageal (RR = 0.56; 95% CI = 0.44–0.71) and gastric cardia (RR = 0.47; 95% CI = 0.33–0.65) cancers. The authors estimated that 26.4% of these cancers in Linxian, China, are attributable to low selenium levels [69]. Experiments in a variety of animal models have demonstrated that selenium can inhibit carcinogenesis [12]. For example, selenium supplied as high-selenium broccoli significantly decreased the incidence of chemically-induced aberrant crypt foci, preneoplastic lesions indicative of colon cancer, in rats [70].

Epidemiological and experimental data suggest that calcium and vitamin D may influence the risk for colorectal and prostate cancers [51]. Numerous epidemiological studies have suggested a weak association between calcium intake and decreased risk for colorectal cancer, but results are not conclusive [5,51,71]. One review of evidence linking dairy products with increased prostate cancer risk and high circulating levels of 1,25-dihydroxyvitamin D (1,25-D) with reduced risk concluded that these relationships may be linked, considering that high levels of calcium and phosphorus from dairy products and sulphur-containing amino acids from animal protein lower the circulating 1,25-D level [72].

Folate and methionine, an essential amino acid, have also been linked to a reduced risk of colorectal cancers and colorectal adenomas in some, but not all, epidemiological studies [5,73,74]. The role of folate in colorectal cancer risk, particularly with regard to the effects of genetic polymorphisms, is discussed in more detail later in this paper.

Based on *in vitro* data, and while recognising their prooxidant potential under certain conditions, some have postulated that anti-oxidant micronutrients may protect against oxidative damage to biomolecules, such as lipids, lipoproteins and DNA, thus influencing the risk for cancer development [49,75,76]. Selenium is a component of numerous selenoproteins (e.g. glutathione peroxidase, thioredoxin reductase) that function as enzymes in redox reactions that may affect cancer risk [77,78]. Anti-oxidant micronutrients may also influence carcinogenesis through other mechanisms. For example, vitamin E inhibits cell proliferation, [79], and caroten-

oids, including β-carotene, may affect cell transformation and differentiation, enhance cell-to-cell communication, and enhance immune responses [49]. Experimental evidence generally suggests that calcium and vitamin D may reduce risk for colorectal cancer by decreasing cellular proliferation [51].

2.4. Phytochemicals

Plant-derived foods, including vegetables, fruits and whole grains, contain thousands of chemically diverse phytochemicals. Many individual phytochemicals have been investigated in laboratory studies to determine their effects on cancer risk and to uncover the mechanisms by which they exert their effects [80–83]. However, the applicability of experimental data on individual phytochemicals to humans is not straightforward; people eat whole foods, which contain numerous phytochemicals that may interact with each other and/or with the micro- and macronutrient components of whole foods. In addition, quantifying intake is a considerable challenge because specific phytochemical content has not been determined for most foods and reliable biomarkers of intake are not generally available. For these reasons, in part, existing data from epidemiological studies investigating a phytochemical–cancer association are difficult to interpret. Furthermore, epidemiological data is lacking for the majority of phytochemicals [5]. The discussion here, although not an exhaustive review, serves to underscore the complexity of the phytochemical–cancer relationship.

Certain phytochemical classes, along with examples of specific compounds, food sources, and representative cancer prevention-related activities are presented in Table 1. Epidemiological information for some classes is presented briefly below, along with some potential mechanisms not included in Table 1.

Common green, yellow/red and yellow/orange vegetables and fruits contain more than 40 carotenoids (e.g. α-carotene, β-carotene, lycopene, lutein and the xanthins) that can be metabolised by humans [100]. Clear evidence of an inverse relationship with cancer risk is not yet available for any of the carotenoids [49]. A recent pooled analysis of cohort data from the NHS and the HPFS indicated that high intakes of α-carotene, lutein, lycopene and β-cryptoxanthin decreased lung

Table 1
Selected phytochemicals associated with cancer prevention[a]

Phytochemical class	Typical compounds	Food sources	Cancer prevention-related activities
Carotenoids	α-Carotene, β-carotene, lycopene, β-cryptoxanthin, lutein, astaxanthin	Yellow–red and dark green vegetables and fruits	Antioxidant activity, modulation of carcinogen metabolism, inhibition of cell proliferation, inhibition of oncogene expression, beneficial effects on immune function, beneficial effects on cell transformation and differentiation, enhancement of cell-to-cell communication
Organosulphur compounds	Diallyl sulphide, diallyl disulphide, allyl methyl trisulphide, dithiolthiones	Sulphides, Allium vegetables (e.g. garlic, onion); dithiolthiones, cruciferous vegetables (e.g. broccoli, cabbage)	Increase phase II enzyme activity, inhibit cell proliferation, induce cell differentiation, alter steroid hormone metabolism, inhibit ornithine decarboxylase activity
Polyphenols	Phenolic acids (e.g. caffeic acid), hydroxycinnamic acids (e.g. curcumin), flavanols (e.g. quercetin, apigenin), flavanones (e.g. naringin, hesperidin), catechins (e.g. epigallocatechin gallate), theaflavins, resveratrol	Vegetables and fruits; catechins, green tea; theaflavins, black tea; resveratrol, red wine	Reduce carcinogen–DNA adduct formation, inhibit cell proliferation, induce cell cycle arrest and apoptosis, inhibit signal transduction pathways, enhance cell-to-cell communication, improve immune function
Phyto-oestrogens	Isoflavones (e.g. genistein, daidzein), lignans (e.g. matairesinol, secoisolariresinol)	Isoflavones, soybeans, soy-based foods; lignans, vegetables, flaxseed, rye	Alter oestrogen metabolism, decrease tyrosine kinase activity, induce cell cycle arrest and apoptosis, induce topoisomerase II-mediated DNA breakage
Glucosinolates, isothiocyanates, indoles	Glucobrassicin, sulphorophane, indole-3-carbinol	Cruciferous vegetables	Increase phase II enzyme activity, induce cell cycle arrest and apoptosis, inhibit cell adhesion and invasion
Terpenes	Monoterpenes (e.g. limonene, perillyl alcohol, geraniol), sesquiterpenes, e.g. farnesol)	Vegetables and fruits (e.g. citrus)	Increase phase II enzyme activity, influence cell cycle progression, induce apoptosis

[a] Information for this table was drawn from Refs. [49,50,83–99].

cancer risk by 25, 19, 20 and 18%, respectively. Smoking attenuated all of the risk reductions except for that of lycopene [101]. One evaluation of 72 studies of tomatoes (high in lycopene), tomato-based products, lycopene, and cancer found the strongest evidence of inverse associations for prostate, lung and stomach cancers [102]. Lycopene has recently been shown to inhibit the action of insulin-like growth factor I (IGF-I) in mammary cancer cells [103].

Epidemiological evidence supports an inverse association with cancer for *Allium* vegetables, rich in organosulphur compounds [5,50]. One review reported that *Allium* vegetables showed an inverse relationship with overall cancer risk in 27 of 35 case–control and cohort studies and an inverse relationship with stomach cancer in 9 of 11 case–control studies [5]. A meta-analysis reported that high intake of raw and cooked garlic may reduce the risk for stomach (RR = 0.53; 95% CI = 0.31–0.92) and colorectal (RR = 0.69; 95% CI = 0.55–0.89) cancers [104]. One recent study on diallyl sulphide (DADS), a frequently investigated *Allium* constituent, in human colon tumour cells suggests that DADS suppresses $p34^{cdc2}$ activity and thus induces a G_2/M phase cell cycle arrest (suppressing cell division) by inhibiting the formation of the $p34^{cdc2}$/cyclin B_1 complex and its subsequent activation [85].

Findings from epidemiological studies that investigated the effects of black (oxidised) or green (unoxidised) tea consumption on cancer risk suggest that green tea consumption may reduce the risk for overall cancer mortality [105]. Although evidence regarding the risk of incidence at specific sites is generally inconclusive [105,106], some data indicate a possible link between tea consumption and a reduced risk for digestive tract cancers [5,107,108]. Most experimental studies examining the tea–cancer relationship have focused on green tea polyphenols (GTP), theaflavins in black tea and extracts of either black or green tea [105,109]. Various possible mechanisms for the cancer-related biological effects of tea have been explored [110]. For example, epigallocatechin gallate (EGCG) and theaflavins block the signalling pathway leading to nuclear transcription factor κB (NF-κB) activation, resulting in antitumour promotion effects [87].

Epidemiological and laboratory data suggest that dietary phyto-oestrogens — that is, isoflavonoids (e.g. genistein, daidzein) and lignans — reduce the risk for certain cancers [111,112]. Soy products have been associated with a decreased risk for breast [113], endometrial [114] and prostate [115,116] cancers. Clinical data suggest that soy diets influence breast cancer risk by favourably modulating oestrogen metabolism in women, thus decreasing the formation of genotoxic 4-hydroxylated oestrogen metabolites [93,117,118]. Prospective data from vegetarian men indicated that consumption of soy milk more than once a day, compared with no soy milk intake, was associated with a 70% reduction in prostate cancer risk [116]. Experimental evidence suggests that dietary soy products may inhibit prostate tumour growth through reduced cell proliferation and angiogenesis and increased apoptosis [119,120].

Identifying valid biomarkers of phytochemical intake will facilitate carrying out future epidemiological studies. For example, glucosinolates and isothiocyanates (ITC) in cruciferous vegetables can be quantified readily in urine as their dithiocarbamate metabolites [121] or as ITC conjugates [122,123]. In a recent study, Chinese men with detectable urinary ITC conjugates had a reduced risk (RR = 0.65; 95% CI = 0.43–0.97) for lung cancer [123].

2.5. Dietary fat

Much research has been conducted on the association of total fat, specific high-fat foods (e.g. meats), and types of either fat or fatty acids and cancer risk. Although data from ecological and animal studies suggest direct associations between total fat intake and increased cancer risk at several sites, including breast, colon/rectum, breast, prostate and lung, findings in analytical studies do not consistently support these associations [5,11]. Given that dietary fat correlates closely with other lifestyle factors, that various types of fat do not contain the same fatty acid profile and that dietary assessment methodologies can introduce significant error, it is not unexpected that linkages are not always evident [124]. Epidemiological data generally support a direct association between colorectal cancer incidence and the consumption of red meat [5,71,125]. Some experimental studies suggest, however, that the increased risk may be linked to the dietary haem in red meat, rather than to fat [126,127], and to the heterocyclic aromatic amines (HAAs) formed in red meat during high-temperature cooking methods, such as frying and broiling [128].

Overall, findings suggest that the link between fat and cancer risk depends on the type of fat consumed rather than, or in addition to, total fat intake. Some evidence suggests that consumption of olive oil may reduce breast cancer risk [129]. Olive oil is rich in oleic acid, a monounsaturated fatty acid (MUFA), and also contains numerous phenolic antioxidants that may have potential for inhibiting carcinogenesis [130]. The cancer-related effects of n-6 polyunsaturated fatty acids (PUFAs), found in common seed oils, and long-chain, n-3 PUFAs, found in fish oils, are of particular interest. Generally, n-6 PUFAs (e.g. linoleic acid) appear to enhance the promotional phase of carcinogenesis in preclinical models for breast, colon and prostate cancers, whereas n-3 PUFAs (e.g. α-linolenic acid, eicosapentanoic acid (EPA), and docosahexanoic acid (DHA)) seem to exert inhibitory effects [11,131]. Epidemiological

and clinical data support a possible inverse relationship between consumption of fish and long-chain, n-3 PUFAs and risk for breast and colorectal cancers [132]. In a European multicentre breast cancer study, the ratio of long-chain, n-3 PUFAs in adipose tissue to total n-6 PUFAs was inversely related to breast cancer risk (RR = 0.65; 95% CI = 0.41–1.03, highest versus lowest tertiles), indicating that the balance between n-3 PUFAs and n-6 PUFAs may be important [133]. These findings agree with mortality data for 24 European countries that show inverse correlations for breast and colorectal cancers with fish and fish oil consumption expressed as a proportion of animal fat [134]. One review of epidemiological and experimental evidence suggested that long-chain, n-3 PUFAs may retard disease progression in prostate cancer [135]. α-Linolenic acid, however, has been associated with almost a 4-fold increased risk of prostate cancer in some studies [136,137]. Although α-linolenic acid is a metabolic precursor of EPA, which is associated with reduced prostate cancer risk, its conversion to EPA in humans is limited [135].

Fatty acids may influence various steps in carcinogenesis through numerous mechanisms that include: peroxidation of PUFAs and subsequent DNA damage [131]; effects on oestrogen concentrations and availability [131]; effects on membrane-bound enzymes that regulate xenobiotic metabolism [131]; alterations in cell membranes, resulting in changes in hormone and growth factor receptors [131]; fatty acid regulation of eicosanoid production and subsequent modulation of immune response [11]; fatty acid activation of nuclear transcription factors (e.g. peroxisome proliferator-activated receptors (PPARs)), leading to cell differentiation [138]; modulation of signal transduction pathways by fatty acids, leading to altered gene expression and effects on cell proliferation and apoptosis [131,139]; and inhibition of translation initiation, leading to decreased cell proliferation because of reduced synthesis and expression of G1 cyclins and cell cycle arrest in G_1 [140].

2.6. Anthropometry/physical activity

Numerous epidemiological studies have investigated the roles of anthropometric measures — including weight or body mass index (BMI), weight gain, height and central adiposity — in relation to cancer risk [5,8]. The strongest evidence indicates a direct association of these anthropometric measures and cancers of the breast, endometrium, colon and kidney [5,8]. For instance, most studies report a doubling or tripling of endometrial cancer risk for women in the highest weight quintile [8]. For colon cancer, findings in a large case–control study indicated that BMI was significantly associated with increased risk in men (RR = 1.96; 95% CI = 1.50–2.57) and in women (RR = 1.45; 95% CI = 1.08–1.94) [141]. Associations with breast cancer have been studied most extensively [8]. In brief, data indicate that rapid growth during adolescence and greater adult height increase risk [5,8]; premenopausal obesity reduces risk during premenopausal years, but increases risk during postmenopausal years [8,142,143]; postmenopausal obesity increases risk [8,143,144]; and central adiposity (large waist-to-hip ratio) increases the risk in both premenopausal and postmenopausal women [8,145]. Adult weight gain appears to be the most consistent measure associated with increased postmenopausal breast cancer risk [8,144,146]. One recent study reported that each 5 kg of weight gain since the lowest adult weight (at age 20 years) increased risk by 8% [146].

The strongest effects of physical activity on cancer risk have been found for breast and colon cancers [5,7,8]. Most studies of physical activity, including both occupational and recreational activity, report a reduction in breast cancer risk in physically active women, although dose–response trends generally are not evident [7,147,148]. For example, 11 of 16 studies on recreational exercise reported a 12–60% decrease in risk [147]. Data from two recent case–control studies in The Netherlands [149] and Switzerland [150] and a cohort study in the United States [151] suggest that physical activity at any age is beneficial. Leanness and regular physical activity have been consistently associated with a reduced risk for colorectal cancer in both men and women. A recent case–control study in Switzerland reported that, in men over age 30 years, those with the highest level of physical activity showed a 37–56% decrease in risk [152].

Mechanisms involving hormones have been proposed to explain the association between body size and physical activity and cancers of the breast, endometrium and colon [8,153,154]. Both overweight and central obesity are associated with increases in oestrogens, which are directly linked to the risk for breast and endometrial cancers [8], and also with increases in insulin and insulin-like growth factors (e.g. IGF-I), which may stimulate cell proliferation and thus increase the risk for breast and endometrial cancers [8,153], as well as for colon cancer [154,155]. Increased exercise and decreased body weight can downregulate IGF-I by increasing the production of IGF-binding protein-1 (IGFBP-1) [153,155]. Recent findings from a cohort study indicated decreased colorectal cancer risk (RR = 0.48; 95% CI = 0.23–1.00) in women in the highest quintile for IGFBP-1 [155].

2.7. Alcohol

Epidemiological data strongly support a direct association of alcohol intake with cancers at several sites — including the aerodigestive cancers (oral cavity, pharynx, oesophagus, larynx) and liver cancer — and suggest a direct association with breast, colorectal, and

lung cancers [5,9]. Reports indicate that alcohol consumption and tobacco use have a multiplicative effect on risk for aerodigestive cancers, with a highly elevated risk observed in heavy smokers [156–158]. For instance, in one study, consumption of $\geqslant 150$ ml ethanol/day and smoking $\geqslant 25$ cigarettes/day both independently increased oesophageal cancer risk (RR = 8.94 and RR = 4.90, respectively); these two behaviours combined, however, increased risk more than 50-fold [156]. Epidemiological data linking alcohol and colorectal cancer are not consistent [5,9]. The effects of alcohol on colorectal cancer risk may depend, in part, on methionine and folate intake. In a large prospective study, men in the highest quintiles of methionine and folate intakes who consumed more than 20 g alcohol daily were not at increased risk for colon cancer (RR = 0.79; 95% CI = 0.38–1.64, RR = 1.03; 95% CI = 0.52–2.06, respectively) [159]. Generally, the association reported between alcohol consumption and breast cancer risk has been modest [9,160]. One meta-analysis of six prospective studies indicated that risk increases linearly with total alcohol intake, and that daily alcohol intake equivalent to 0.75–1 drink is associated with a 9% increase in risk [160]. Several recent prospective studies have found no association of light alcohol consumption with breast cancer risk [161,162], but some data suggest increased risk at higher levels of intake [162,163]. It has been proposed that, in certain women, hyperinsulinaemia resulting from long-term, moderate alcohol consumption can stimulate expression of IGF-I receptor in mammary tissue, which could accelerate oestrogen-independent growth in precancerous lesions [164].

Possible mechanisms by which alcohol might increase overall cancer risk include carcinogenicity of acetaldehyde, a metabolite of alcohol; carcinogenicity of congeners in alcoholic beverages (i.e. preservatives, flavouring agents); effects on cell membrane integrity; increase in lipid peroxidase; effects on carcinogen metabolism; alteration of hormone levels, particularly oestrogens; and impairment of nutrient metabolism [9,165]. For instance, in rats, high alcohol intake can lead to folate deficiency in the colon, likely a result of folate breakdown by acetaldehyde produced by microbial oxidation of alcohol [166].

3. Diet and cancer prevention clinical trials

Randomised, controlled dietary intervention and chemoprevention trials to test hypotheses generated from epidemiological and laboratory investigations on diet and cancer prevention are designed to answer questions related to the ability of dietary patterns and dietary constituents to prevent cancer (primary prevention) or its recurrence (secondary prevention). Such trials are relatively recent additions to the cancer research armamentarium and represent two avenues of attack for preventing neoplasm initiation and progression. Although dietary intervention and chemoprevention trials share the goal of determining how diet can affect the diet–cancer relationship, they differ in the methodologies and the activities used to achieve that goal. Dietary intervention trials investigate the effects on cancer risk of modifying the intake of either whole foods, such as vegetables, fruits, and grains, or macronutrients, such as fat and fibre. Generation of hypotheses based on epidemiological and experimental studies is followed by development and testing of methods to determine whether dietary interventions can, in fact, be implemented and dietary intake accurately validated. Subsequently, controlled feeding/metabolic studies may be used to assess the effect of discrete dietary changes on intermediate or disease outcomes. If a hypothesis is supported by controlled feeding/metabolic studies, a phase III study — a randomised clinical trial in a large number of people with incidence as an endpoint — is conducted to determine whether the specific intervention actually reduces cancer risk. Chemoprevention trials investigate the abilities of specific dietary constituents (e.g. vitamins, minerals, phytochemicals) or synthetic compounds (e.g. pharmacological agents) to block or suppress the initiation or progression of carcinogenesis. Chemoprevention trials test potential cancer-inhibitory agents in initial phase I (pharmacological and toxicological profile) and phase II (biomarker endpoint) trials to determine which agents have the most potential with regard to high efficacy and low toxicity. If findings in phase I and II clinical studies of a chemoprevention agent support the initial hypothesis, a phase III study is conducted to determine specific clinical outcomes.

3.1. Ongoing phase I and II chemoprevention trials

Chemoprevention research opportunities have expanded in the past decade as our understanding of carcinogenesis has increased. The US National Cancer Institute (NCI) has an aggressive chemoprevention programme based on findings from epidemiological and experimental research. Further, there are indications from several international groups (e.g. International Union Against Cancer, European Union) that chemoprevention may become a stronger research focus in Europe [167]. Further, chemoprevention research is ongoing in China (i.e. Linxian study) and is emerging in Japan, where the focus is on hepatocellular carcinoma, gastric cancer and colon cancer [168]. Of particular interest to chemoprevention research in the US are agents that have been found to be antimutagenic (e.g. calcium, indole-3-carbinol), antiproliferative (e.g. perillyl alcohol, selenium, soy isoflavones), and antiangiogenic (e.g. retinoids, protease inhibitors) [169]. Table 2 lists selected chemoprevention agents currently being evaluated

Table 2
Selected NCI phase I and II chemoprevention trials using dietary constituents

Phase I			Phase II		
Dietary factor	Target organ	Patients *n*	Dietary factor	Target organ	Patients *n*
Curcumin	Colon	36	9-*cis*-Retinoic acid	Cervix	74
Perillyl alcohol	Breast	30	Indole-3-carbinol	Anogenital warts/HPV	200
Perillyl alcohol	Breast	24	Perillyl alcohol	Breast	45
Soy isoflavones	Prostate	24	Vitamin D + calcium	Colon	40
Soy isoflavones	Breast	24	Topical polyphenon E (wartheal)	Skin (actinic keratosis)	60
Lycopene (Tomato paste) — three trials	Prostate	75 (25 each)	Soy isoflavones	Prostate (presurgical)	80
Epigallocatechin-gallate (EGCG) and polyphenon E	Skin	40			
Soy isoflavones	Prostate	12			

HPV, human papilloma virus.

in phase I and II trials at the NCI, where more than 40 agents and agent combinations are being investigated for major cancer sites, including breast, prostate, colon and lung. For example, soy isoflavones are being investigated in phase I trials as selective oestrogen receptor modulators with possible application in the chemoprevention of breast and prostate cancers, and lycopene is being investigated for its ability to inhibit carcinogen-induced aberrant crypt foci in the colon [169,170]. Phase II trials often are designed to identify intermediate (or surrogate) endpoints that are on the pathway for carcinogenesis and that can be modulated by the intervention; however, such intermediate endpoints must be well-defined and validated to be useful [171].

3.2. Completed and ongoing phase III cancer prevention trials

Phase III randomised, controlled trials of dietary interventions and chemoprevention are the best method for testing hypotheses relevant to the diet–cancer relationship. Examples of completed and ongoing large-scale dietary intervention and chemoprevention phase III clinical trials are listed in Table 3. As seen from the results of these trials, progress is being made in determining the roles of dietary factors in cancer prevention. Even when associations between dietary constituents and cancer risk are not confirmed in phase III trials, much can be learned. For example, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) was one of the first large-scale randomised, controlled chemoprevention trials to test the hypothesis that vitamin E and β-carotene could reduce lung cancer risk. After more than 5 years of follow-up, however, data indicated an increase in the incidence of lung cancer among current and former smokers who received β-carotene supplements [57]. Although the original hypothesis in the ATBC Study was not confirmed, secondary endpoint analyses of follow-up data found decreases of 32 and 41% in prostate cancer incidence and mortality, respectively, among current and former smokers receiving vitamin E, providing leads for future research [172]. Similarly, in the Nutritional Prevention of Cancer Trial (NPCT), even though data did not support the primary endpoint of a reduction of non-melanoma skin cancer, a secondary endpoint analysis indicated that selenium may reduce the incidence of prostate cancer [173]. These results from the ATBC Study and the NPCT have led to the design of the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a large-scale randomised, controlled phase III trial to investigate further the role of selenium and vitamin E in prostate cancer prevention.

4. Emerging evidence: gene–nutrient interaction

Numerous types of genes are likely to be involved in human carcinogenesis, including genes that influence metabolic activation/detoxification, DNA repair, chromosome stability, activity of oncogenes or tumour suppressor genes, cell cycle control, signal transduction, hormonal pathways, vitamin metabolism pathways, immune function and receptor or neurotransmitter action [4]. Understanding how nutrients and other diet-related factors can inhibit or promote the carcinogenic process through interactions with various genes is essential — to facilitate both the interpretation of data provided by ongoing studies and the development of effective strategies for cancer prevention. During the past decade, interest in gene–nutrient interactions has grown considerably, and this emerging research area shows great promise as a means to further progress in the overall reduction of cancer risk. To illustrate the relevance of gene–nutrient interactions to cancer research, some examples of such interactions, including the role of genetic polymorphisms, are presented below.

Dietary carcinogens such as aflatoxin B_1 (AFB_1), HAAs and polycyclic aromatic hydrocarbons (PAHs) can alter DNA by forming adducts [182]. In fact, urin-

Table 3
Large-scale phase III cancer prevention trials[a]

Trial	*n*	Follow-up/endpoint	Intervention	Relative risk for intervention	
				Mortality	Incidence
Skin Cancer Prevention Study [174]	1805 adults in US	5 years. Incidence of non-melanoma skin cancer. 8.2 years. Mortality, all sites	50 mg β-carotene	0.8 (0.5–1.3), all sites	1.0 (0.9–1.2), skin 1.0 (0.9–1.2), basal 1.2 (0.9–1.7), squamous
Linxian, China [175]	29 584 adults in China	5.25 years. Cancer mortality, all sites, oesophageal, stomach	5000 IU retinol and 22.5 mg zinc 3.2 mg riboflavin and 40 mg niacin 120 mg vitamin C and 30 mcg molybdenum 15 mg β-carotene, 50 mcg selenium and 30 mg α-tocopherol	1.0 (0.9–1.1), all sites 0.9 (0.8–1.2), oesophagus 1.0 (0.8–1.3), stomach 1.0 (0.9–1.1), all sites 0.9 (0.7–1.1), oesophagus 1.0 (0.8–1.2), stomach 1.1 (0.9–1.2), all sites 1.1 (0.9–1.3), oesophagus 1.1 (0.9–1.4), stomach 0.9 (0.8–1.0), all sites 1.0 (0.8–1.2), oesophagus 0.8 (0.6–1.0), stomach	
Linxian, China [176]	3318 adults in China with oesophageal dysplasia	6 years. Cancer mortality and incidence, all sites, stomach, oesophageal	High dose (2–3 times the RDA) multivitamin with minerals, including 15 mg β-carotene	1.0 (0.7–1.3), all sites 1.2 (0.8–1.9), stomach 0.8 (0.5–1.3), oesophagus	1.0 (0.8–1.2), all sites 1.2 (0.9–1.6), stomach 0.8 (0.7–1.2), oesophagus
The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study [57]	29 133 male smokers in Finland	5–8 years. Cancer mortality, lung, other	20 mg β-carotene	1.2 (ns), lung 1.0 (ns), other	1.2 (1.0–1.4), lung 1.0 (ns), bladder 1.0 (ns), colorectal 1.2 (ns), prostate 1.3 (ns), stomach 0.9 (ns), other
		Cancer incidence. Lung, bladder, colorectal, prostate, stomach, other	50 mg α-tocopherol	1.0 (ns), lung 1.1 (ns), other	1.0 (0.9–1.1), lung 1.1 (ns), bladder 0.8 (ns), colorectal 0.7 (0.001), prostate 1.3 (ns), stomach 1.1 (ns), other
Beta Carotene and Retinol Efficacy Trial (CARET) [58]	18 314 former and current smokers; US asbestos workers	4 years. Mortality, lung. Incidence, lung, prostate, other cancers	3 mg β-carotene and 25 000 IU retinol	1.5 (1.1–2.0), lung	1.3 (1.0–1.6), lung No association, prostate No association, others
Nutritional Prevention of Cancer Trial [68]	1312 History of basal cell or squamous cell carcinomas of the skin	6.4 years.	200 mcg selenium supplied as a 0.5 g high-selenium brewer's yeast tablet	0.5 (0.3–0.8), all sites	0.6 (0.4–0.8), total 1.1 (1.0–1.3), basal 1.1 (0.9–1.4), squamous 0.5 (0.3–1.0), lung 0. 4 (0.2–0.7), prostate

(continued)

Table 3 (*continued*)

Trial	*n*	Follow-up/endpoint	Intervention	Relative risk for intervention	
				Mortality	Incidence
European Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer (EUROSCAN) [177]	2592 smokers in Europe	2 years. Head, neck, and lung cancer mortality and recurrence	N-acetylcysteine retinyl palmitate	1.1 (0.9–1.3), all sites 1.0 (0.9–1.2), all sites	1.0 (0.9–1.2), all sites 1.0 (0.9–1.2), all sites
Women's Health Study [60]	39 876 women aged 45 years or older	2.1 years	50 mg β-carotene on alternate days	1.1 (0.7–1.8), all sites	1.03 (0.9–1.2), all sites
Polyp Prevention Trial I [178]	864 patients with adenoma	4 years. Occurrence of new adenomas	25 mg β-carotene 1 g vitamin C and 400 mg vitamin E		1.01 (0.85–1.20) 1.08 (0.91–1.29)
Physicians' Health Study I [59]	22 011 male physicians in US	12 years. Cancer mortality, all sites. Cancer incidence, all sites	50 mg β-carotene on alternate days	1.0 (0.9–1.2), all sites No association with cancer of the bladder, colorectal, lung, brain, prostate, pancreas, melanoma, leukaemia, or lymphoma	1.0 (0.9–1.1), all sites No association with cancer of the bladder, colorectal, lung, brain, prostate, pancreas, melanoma, leukaemia, or lymphoma
Australian Polyp Prevention Project [41]	411 patients with previous colorectal adenoma	4 years. Incidence of adenoma, any grade	25 g wheat bran (11 g dietary fibre) 20 mg β-carotene		1.5 (0.9–2.4), 24 months 1.5 (0.9–2.5), 48 months 1.4 (0.8–2.3), 24 months 1.3 (0.8–2.2), 48 months
Polyp Prevention Trial II [40]	1905 patients with previous adenomas	4 years. Adenoma recurrence	Low-fat, high-fibre, high-vegetable and fruit diet with behaviour modification		1.00 (0.90–1.12)
European Cancer Prevention Calcium Fibre Polyp Prevention Study [179]	655 patients with previous adenoma	3 years. Follow-up at 6 month intervals. Adenoma recurrence	calcium fibre (ispaghula husk)	Ongoing	
Physicians' Health Study II [180]	~15 000 physicians in US	5 years. Total and prostate cancer	vitamin E vitamin C multivitamins 2×2×2×2 factorial design	To begin in 2000/2001	
Selenium and Vitamin E Cancer Prevention Trial (SELECT)	32 000 men anticipated	7 years (trial to be completed in 12 years)	selenium vitamin E both	To begin recruitment phase in 2001	

ns, non-significant.

[a] Adapted from Patterson and colleagues, Table 1 [181].

ary excretion of AFB_1–DNA adducts is sometimes used as a biomarker of aflatoxin exposure and liver cancer risk. A nested case–control study in Shanghai noted that the presence of urinary AFB_1–DNA adducts was associated with a 3.4-fold increase in liver cancer incidence [183]. Dietary aflatoxin consumption, assessed by a food frequency interview, did not reflect this association, emphasising the importance of biomarker measurements. Recent studies reported that a diet rich in vegetables, fruits and cereals was associated with a reduction in white blood cell PAH–DNA adducts [184], and that consumption of carotenoid-rich carrot and tomato juices reduced oxidative DNA damage in human lymphocytes by various mechanisms [185], possibly reducing cancer risk. Further, experimental data indicate that n-3 and n-6 PUFAs are involved in gene regulation and transcription, mRNA stability and cellular differentiation in the normal cell, [186], and that n-6 PUFAs affect the expression of tumour suppressor genes [187].

Polymorphisms with differing activities exist for many genes involved in the metabolism and detoxification of carcinogenic substances, including the P450 genes for the cytochrome P450 phase I enzymes that catalyse the oxidative metabolism of endogenous substances (e.g. fatty acids, steroids) and exogenous chemicals (e.g. HAAs, PAHs); and the genes for the phase II enzymes (epoxide hydrolase, GST, *N*-acetyltransferase (NAT), sulphotransferase) that detoxify carcinogenic metabolites by producing readily-excreted, hydrophilic conjugation products [188]. A comprehensive review of the molecular genetics and epidemiology of *NAT1* and *NAT2* polymorphisms concluded that these polymorphisms modify risks for developing urinary bladder, colorectal, breast, head and neck, lung and possibly prostate cancers [189]. Associations between slow *NAT2* acetylator genotypes and urinary bladder cancer and between rapid *NAT2* acetylator genotypes and colorectal cancer are most consistently reported.

Many other genetic polymorphisms may have relevance for diet and cancer, such as those governing the expression of alcohol dehydrogenase 3 (ADH_3) [190], manganese superoxide dismutase (MnSOD) [191], methionine synthase (MS) [192] and methylenetetrahydrofolate reductase (MTHFR) [193,194]. One case–control study found more than a 3.5-fold increase in breast cancer risk in premenopausal, but not postmenopausal, women with the ADH_3^{1-1} genotype (the rapid form of the enzyme) who had a lifetime alcohol consumption higher than the median [190]. In another case–control study that characterised *MnSOD* genotypes in relation to breast cancer risk, premenopausal and postmenopausal women who exhibited the AA genotype had 4.3- and 1.8-fold increases in breast cancer risk, respectively, compared with the AV or VV genotypes. Interestingly, risk was greatest (OR = 6.0; 95% CI = 2.0–18.2) for premenopausal women below the median consumption of vegetables, fruits and dietary antioxidants [191].

Folate–gene interactions provide excellent examples to illustrate the importance of investigating diet and cancer relationships at the molecular level. The possible relationship of dietary folate with both hypomethylation and hypermethylation of DNA and the consequent putative role of folate in determining risk for certain cancers has received considerable attention [53,195,196]. MTHFR is a critical enzyme that regulates the metabolism of folate, a key compound in DNA metabolism and synthesis. The enzyme irreversibly converts 5,10-methylenetetrahydrofolate (methyleneTHF), the major form of intracellular folate and the cofactor for methylating dUMP to dTMP in deoxynucleotide synthesis, to 5-methyltetrahydrofolate (methylTHF), the major form of circulating folate in plasma. A common polymorphism of the *MTHFR* gene (677C→T) results in an alanine→valine substitution in the enzyme and, consequently, an enzyme with significantly decreased activity [193,197,198]. The decreased activity of the MTHFR enzyme increases methyleneTHF at the expense of methylTHF; increased availability of methyleneTHF for DNA synthesis reduces the chances for insufficient methylation of dUMP to dTMP as well as for incorporation of uracil into DNA. Reduced incorporation of uracil into DNA leads to fewer chromosome breaks and possibly less cancer risk [198,199]. Studies using data from the HPFS [200] and the Physician's Health Study (PHS) [201] on the interaction of the 677C→T *MTHFR* polymorphism and dietary intake of folate and methionine (sources of methyl groups) in colorectal tumorigenesis found that, when the dietary methyl supply was high, MTHFR *val*/*val* individuals (low enzymatic activity) were at reduced risk of colorectal cancer (RR = 0.57; 95% CI = 0.30–1.06 and RR = 0.46; 95% CI = 0.25–0.84, respectively). However, when the dietary methyl supply was either low or depleted because of alcohol consumption, the adverse association of alcohol with colorectal cancer was slightly stronger among *val*/*val* individuals in both HPFS (val/val, RR = 1.56; 95% CI = 0.65–3.81; val/ala and ala/ala, RR = 1.35; 95% CI = 0.82–2.24) and PHS (val/val, RR = 1.31; 95% CI = 0.48–3.58; val/ala, RR = 1.00; 95% CI = 0.51–1.94; ala/ala, RR = 0.72; 95% CI = 0.37–1.42), suggesting that individuals with this genotype may be more sensitive to the carcinogenic effect of alcohol [193,200,201]. A recent study that used HPFS and PHS data to investigate the relationship of a polymorphism (2756AG, aspgly) in the gene for MS, another important enzyme in folate metabolism, and colorectal cancer risk reported an overall inverse risk association for the *gly*/*gly* genotype (RR = 0.59; 95% CI = 0.27–1.27), compared with the *asp*/*asp* genotype [192]. Similar to MTHFR, data indicated an interaction between alcohol intake and the *MS* genotype; men with the *gly*/*gly* genotype who consumed

<1 drink/day were at lower risk for colorectal cancer (RR = 0.27; 95% CI = 0.09–0.81) than those who consumed $\geqslant 1$ drink/day (RR = 2.64; 95% CI = 0.65–10.82). The level of alcohol intake did not affect risk for *gly*/*asp* and *asp*/*asp* genotypes [192].

Methyl-deficient diets may also contribute to breast cancer risk. Recent studies have shown that oestrogen receptor (ER)-negative breast cancer results from a lack of *ER* gene expression caused by methylation of the CpG island 5′ to the gene [196]. CpG island methylation appears to be an early event in carcinogenesis. Thus, it is hypothesised that diets either deficient in methyl groups or high in methyl-group antagonists (such as alcohol) might cause increased DNA methyltransferase activity, which could increase methylation of usually unmethylated CpG sites, suppressing activities of the *ER* gene, and possibly increasing cancer risk [196]. Furthermore, it is hypothesised that the effect of methyl-deficient diets on breast cancer through methylation of *ER* genes might be modified by the *MTHFR* genotype [196]. However, if ER-positive breast cancer (without methylated *ER* genes) has a differing aetiological pathway, risk factors and appropriate preventive measures for this form of the disease may also differ.

5. Future research directions: a new paradigm

Data from epidemiological, preclinical and clinical intervention studies have contributed tremendously to the extensive body of evidence linking diet with cancer prevention. However, we have only begun to scratch the surface. Current knowledge of the underlying basis of diet–cancer relationships is minuscule. Scientific and technological advances have reached the point where it should be possible to move beyond studies that merely quantify diet and cancer associations to basic research studies that strive to understand cause and effect by investigating the events in molecular biology and genetics that are important to diet-related carcinogenesis. This approach should complement epidemiological and metabolic cancer research designed to search for preventive or adverse effects of particular dietary constituents and to take into account interindividual differences in genetic susceptibility. As an example, the prevalence of genetic polymorphisms in seven of nine genes tested in a random sample of individuals in the ATBC Study — including ADH_3, *GST*, *MS* and *MTHFR* — demonstrate substantial variability in genetic susceptibility in this Finnish population, which by other standards is considered relatively homogeneous [202]. These findings indicate that stratifying the ATBC Study population, based on genotype, and conducting nested case–control studies could provide valuable information on possible gene–nutrient interactions.

Information from various types of investigations will continue to have an important role in clarifying the complex relationship between diet and cancer. However, greater attention to linkages between diet and genetics will enhance the opportunities for developing effective intervention strategies for cancer prevention. The future for understanding diet-and-cancer linkages will be expanded by the ability of the biomedical research community to use newly available technological advances to conduct basic research studies in molecular biology and genetics. This expanded approach for diet, gene and cancer research is not simple; it has many implications and certainly cannot be implemented overnight. It will take motivation, dedication, collaboration and education and training across disciplines, as well as a concerted effort by nutritional scientists, molecular biologists, geneticists and clinical cancer researchers to achieve this vision. Although implementing such a paradigm admittedly will be a tremendous challenge, it is anticipated that the results will be exceptional and will move the research field of diet and cancer into a vitally important position in the battle against cancer. Further, we envision that, during the early decades of the 21st century, researchers in diet and cancer will keep the best of the 'old' science and, using the new paradigm, combine it with the best of the 'new' science, to design effective, targeted cancer prevention strategies that will benefit both the general population and those at high risk for cancer.

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