Oncogene (2004) 23, 6349–6364

www.nature.com/onc

Diet and cancer prevention

Marjorie L McCullough*,[1] and Edward L Giovannucci[2,3]

[1]*Epidemiology and Surveillance Research Department, American Cancer Society, 1599 Clifton Road, NE, Atlanta, GA, USA;* [2]*Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA, USA;* [3]*Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA*

Dietary effects are presumed to underlie many of the large international differences in incidence seen for most cancers. Apart from alcohol and a few micronutrients, however, the role of specific nutritional factors remains ill-defined. The evidence for a role of energy balance, physical inactivity, and obesity has strengthened, while for dietary fat it has weakened. Phytochemicals such as folate, lycopene and flavonoids are still the subject of active research. As the mechanisms underlying human carcinogenesis are better understood, dietary research will focus increasingly on intermediate markers such as the insulin-like growth factors and potentially carcinogenic metabolites.
Oncogene (2004) **23,** 6349–6364. doi:10.1038/sj.onc.1207716

Keywords: diet; nutrition; neoplasms; prevention

Introduction

Nutritional factors have been estimated to contribute to 20–60% of cancers worldwide and to approximately one-third of deaths from cancer in Western countries (Doll, 1992). Many studies have attempted to support, clarify, and add to the hypothesized nutritional factors presented in Doll and Peto's landmark 1981 report (Doll and Peto, 1981). However, with the exception of accumulating evidence that obesity, physical inactivity, alcohol, and probably folate and calcium contribute to cancer, the specific nutritional factors that increase or decrease the risks of human cancer are still largely unknown.

The study of nutritional factors that contribute to cancer has advanced more slowly than have nutritional studies of some other common chronic diseases such as heart disease for several reasons, most importantly the need for validated intermediate risk factors or biomarkers for cancer (Schatzkin and Gail, 2002; Branca *et al*., 2001). In the absence of measures such as specific lipid fractions or blood pressure that are direct determinants for heart disease development, we must rely primarily on measuring cancer outcomes, which may take decades to develop. Intervention trials of cancer development take years, perhaps decades, to test a limited set of nutritional hypotheses, and thus are often cost-prohibitive. Further, because critical exposure periods are not usually known, determining when nutritional factors may act is difficult, and most studies only measure dietary intake at one point in time. Another consideration is that each cancer site or even subtype within the same site may have distinct etiologies. Thus, studying nutritional factors in cancer has been challenging, but several hypotheses of causal relationships are gaining support.

Owing to its broad scope, we limit this review mainly to human studies, and primarily to cancers that are common in developed countries, giving stress to findings from prospective cohort studies because these are less prone to bias than retrosopective studies.

*Correspondence: ML McCullough;
E-mail: marji.mccullough@cancer.org

Energy balance, insulin, and insulin-like growth factor (IGF)-I

Energy restriction unequivocally reduces tumor development in animal models (Hursting *et al*., 2003), but to study energy intake in relation to human cancer is complicated. In free-living human populations, variation in energy intake is determined by physical activity, body size, metabolic efficiency, and energy balance (Willett, 1998). Each of these factors could possibly be related to cancer risk. Energy intake and expenditure (i.e. energy balance) is difficult to assess directly in large-scale epidemiologic studies, but measures of body weight and height can serve as proxies for net energy balance over time and energy availability during growth, respectively. Tallness has been consistently associated with increased risk of breast, colon, prostate, and other cancers, and obesity has been associated with a higher risk of postmenopausal breast, colon, uterine, pancreatic, kidney, and certain other cancers (Albanes *et al*., 1988; Willett, 1998; Calle *et al*., 2003). In addition, physical inactivity has been consistently related to a higher risk of colon, breast, and possibly other western cancers (Friedenreich, 2001), although the dose–response relation and relevant exposure periods need clarification. The fact that positive associations for many cancers common in western countries are observed with obesity (surrogate of excess energy

consumption), low physical activity (surrogate of energy expenditure), and taller height (surrogate of total lean body mass and high energy intake during development) makes a compelling case that excess energy balance throughout life is a critical factor for many cancers common in developed countries.

At least part of the effect of excess energy is thought to be mediated through the insulin and IGF pathways (Figure 1). In animal models, a reduction in IGF-I is critical to the cancer-lowering effects of caloric restriction (Hursting *et al*., 2003). IGF-1 inhibits apoptosis, is required for cell cycle progression, and influences renewal of cell populations in several organs (Pollak, 2000). Elevated levels of both insulin and IGF, for which energy balance is the major determinant, have been related to an increased risk of colorectal, prostate, and premenopausal breast cancer (Yu and Rohan, 2000; Giovannucci, 2001; Pollak, 2001). The animal data on energy restriction, the growth-promoting effects of insulin and IGF-1, the human data on insulin and IGF-1 levels and on obesity, tallness, and physical inactivity as risk factors for Western-related cancers all support the hypothesis that energy balance is a critical carcinogenic factor. In fact, arguably, the high consumption of energy intake relative to needs in Western countries where access to energy-dense foods is widespread, and physical exertion levels are low, may be the most important nutritional factor related to cancer in these countries. As these issues related to energy intake and obesity are addressed in this issue by Calle and Thun, they will not be considered further here, and instead we focus on the influence of nutrients, foods or dietary patterns independent of their effects on energy balance.

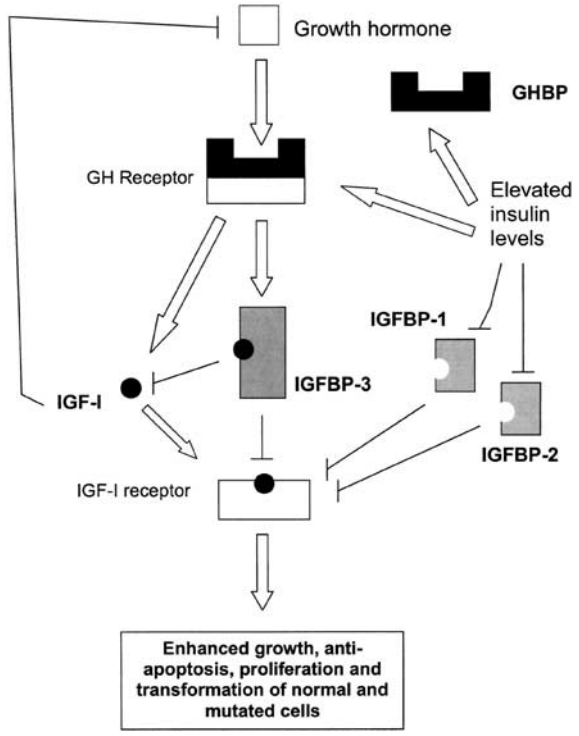

Figure 1 Biologic interactions at the pituitary and hepatic levels among insulin, growth hormone (GH), IGF-I, and insulin-like growth factor-binding proteins (IGFBPs). Open arrows denote stimulation, and thin black lines denote inhibition. Elevated insulin levels (at right) may indirectly increase the bioavailability of IGF-I (solid circles) by suppressing the production of IGFBP-1 and, to a lesser extent, IGFBP-2 (shaded symbols). In turn, increased IGF-I bioavailability may increase negative feedback effect on GH (open square), leading to reduced GH secretion and lower hepatic production of IGF-I and IGFBP-3. However, elevated insulin levels may also increase hepatic GH receptor (open bar) number and activity, reflected by increases in levels of circulating growth hormone-binding protein (GHBP). This effect may lead to a rise in GH-regulated hepatic IGF-I and IGFBP-3 production, with a greater increase in levels of circulating IGF-I relative to IGFBP-3 (Sandhu *et al*., 2002)

Macronutrients

Carbohydrates, protein, and fat each contribute energy to the diet, so excess energy intake resulting from overconsumption of any or a combination of these macronutrients may influence cancer risk. However, the question of whether individual macronutrients increase risk independent of energy balance remains controversial. For example, in intervention studies of low-fat diets under isocaloric conditions, calories from fat are replaced with calories from carbohydrate and/or protein. Other nutrients are likely to change as well. As a result, differentiating the effect of reduced fat intake from these other changes is difficult. Likewise, simply adding fat to the diet cannot be separated from adding energy (or the consequent weight gain). Epidemiologic studies of macronutrients must therefore carefully consider total energy and other energy-bearing nutrients in the analysis (Willett and Stampfer, 1986).

Fat

The results of studies of fat consumption in relation to cancer have been inconsistent, particularly across study designs. Much of the interest regarding nutrition and cancer originated from ecologic studies, in which countries with high per capita fat intake were shown to have higher rates of cancers of the breast (Carroll, 1975), colon, and prostate (Armstrong and Doll, 1975) than countries with lower fat intakes. As important confounders were not measured and controlled for in these analyses, dietary fat may also have been merely a marker for a true causal factor (e.g. reproductive factors). Animal studies also contributed to the hypothesis that fat caused cancer but fat intake was likely to be a surrogate of energy intake (Willett, 1999). Fat intake has been hypothesized to increase the risk of breast and prostate cancer by modulating sex hormone levels, and to increase colon cancer risk by stimulating mutagenic secondary bile acid secretion. Early dietary guidelines, based on these data, emphasized fat reduction for cancer prevention (Palmer, 1983).

Despite the initial excitement, confirming a role for fat, independent of calories and other constituents of meat or dairy foods, or other potential risk factors in colon and breast cancer development, has been difficult.

Colorectal adenomas and cancer have not generally been related to fat consumption in case–control (Howe *et al*., 1997), prospective (Giovannucci and Goldin, 1997; Potter, 1999) or intervention studies (Schatzkin *et al*., 2000). Prospective cohort studies of breast cancer have likewise not supported a role of dietary fat, as was suggested in ecologic and some case–control studies (Smith-Warner *et al*., 2001a; Kushi and Giovannucci, 2002). The major concern with case–control studies is whether diet is recalled differently among those with disease, compared to those without disease. To test whether this potential problem may influence epidemiologic studies of fat and breast cancer, a case–control study was mimicked (Giovannucci *et al*., 1993) within the prospective Nurses' Health Study cohort, where previous measures of diet had been collected. These authors observed positive associations between total and saturated fat and breast cancer risk when using diet reports completed after diagnosis, but not with the earlier, prediagnostic reports. In this case, the relation between fat and breast cancer was overstated using the case–control approach. A pooled analysis of data from eight prospective cohorts with over 7000 breast cancer cases did not observe an important role of fat intake in adult life and breast cancer incidence (Smith-Warner *et al*., 2001a).

In contrast, a European prospective study including 168 cancer cases recently reported a doubling in the risk of breast cancer with high compared to low saturated fat intake (Bingham *et al*., 2003b). As this study observed a significant positive association only with 7-day food records, but not with a food frequency questionnaire (FFQ), the question of imprecision in assessing dietary fat through FFQs was raised. However, both dietary assessment methods have advantages and disadvantages, as reviewed in detail elsewhere (Bingham, 1987; Margetts and Nelson, 1997; Willett, 1998). It is unlikely that recording 7 days of diet (to assess long term exposure) would provide sufficiently superior data to FFQs, such that an association would be detected with 168 cases but entirely missed with over 7000 cases using FFQs. A recent study (using an FFQ) reported a positive association between animal fats consumed earlier in adult life and breast cancer risk (Cho *et al*., 2003b). Thus, it may be that adult diet later in life may have less impact on postmenopausal breast cancer, whereas exposures earlier in life may play a stronger role.

Some of the inconsistencies in fat and breast cancer may be resolved when results from a large, ongoing intervention trial of a low-fat diet in women, the Women's Health Initiative (Rossouw *et al*., 1995), is completed. However, the increased intake of fruits, vegetables, and grains (Rossouw *et al*., 1995) will complicate the interpretation of results. Some of the inconsistencies would also be resolved through use of dietary biomarkers in lieu of questionnaires. Although biologic assessment of blood, adipose tissue, urine, stool or other biologic material is often used effectively as an objective marker of dietary exposures, no established biomarker currently exists for total fat intake. Biomarkers of dietary intake are not the total answer and most available markers are subject to marked interindividual variation, and often reflect only recent intakes; quantification may also be extremely complex (Crews *et al*., 2001).

Evidence has been stronger for a role of animal fats in prostate cancer (Clinton and Giovannucci, 1998; Kolonel *et al*., 1999), but associations appear to vary by the type of fat and study design. Fat from red meat (mostly beef) and dairy sources has been related to a higher risk of prostate cancer in many though not all studies, possibly implicating fatty acids unique to these sources. Marine fatty acids and their food sources (fish) may reduce breast and prostate cancer risk, although findings are inconsistent and more detail on intake of specific fatty acids is needed (Terry *et al*., 2003b). Thus, while some aspect of fat consumption in early or older adulthood may still be positively related to certain cancers including prostate cancer, any associations are much weaker than originally assumed.

Carbohydrates

Coincident with the strong emphasis on lowering dietary fat over the past several decades, grain and sugar consumption in the US increased markedly (Enns, 1997). Carbohydrates are heterogenous and probably have varying effects on health and disease. Dietary carbohydrates include starches (e.g. bread, pasta, other grains), non-starch polysaccharides (the major component of dietary fiber), and sugars. Carbohydrates with a high glycemic index (Wolever and Jenkins, 1986) are associated with higher postprandial blood glucose and insulin (Miller, 1994), and higher fasting insulin levels in insulin-resistant states (Pereira *et al*., 2002), and are thus hypothesized to increase cancer risk. However, epidemiologic studies currently provide limited support for a direct role of diets high in glycemic load (which takes the total carbohydrate intake into account) in cancer development. Some studies of colon (Terry *et al*., 2003a) and breast cancer (Jonas *et al*., 2003) did not find an association between diets high in glycemic load or sugar and cancer, while others did (Slattery *et al*., 1997; Augustin *et al*., 2001; Higginbotham *et al*., 2004). The inconsistencies may result from difficulties in measuring the glycemic potential of diet, given the importance of meal composition, for example. However, glycemic load measured from FFQs has strongly predicted the risk of coronary heart disease (Liu *et al*., 2000) and type II diabetes (Salmeron *et al*., 1997). Glycemic load may increase the risk particularly among susceptible subgroups; one study observed increased risk of breast cancer only among those with elevated BMI (Cho *et al*., 2003a). Pancreatic cancer risk was increased by 53% with a high glycemic diet in a study of women, and by 170% among those who were sedentary and overweight (Michaud *et al*., 2002). More work is needed, as most studies of carbohydrates and cancer risk have not considered carbohydrate quality. Nonetheless, the existing data suggest that abnormal glucose and insulin metabolism is important in carcinogenesis, especially in obese, sedentary individuals.

Protein

Most epidemiologic studies suggest that a high protein intake by itself, at least in adulthood, is unlikely to influence the risk of cancer (World Cancer Research Fund & American Institute for Cancer Research, 1997). However, protein source may be important for various reasons, as described in the *meat* and *dairy* sections below.

Specific foods

Meat intake

Evidence for a role of meat consumption in increasing cancer risk, especially of the colon, rectum, and prostate, has been fairly consistent over time and across study designs. Countries with high per capita meat consumption were shown to have higher incidence of colon cancer than those with low meat consumption, as shown in Figure 2. Two meta-analyses of meat and colorectal cancer risk were recently published, one including 13 prospective studies (Sandhu *et al*., 2001), and the other including results from 34 case–control studies and 14 prospective cohort studies (Norat *et al*., 2002). The former reported a significant 12–17% increase in risk associated with each daily 100 g increment of all meat or red meat intake (slightly more than 3 oz), and a 49% increased risk for each 25 g increment of processed meats (about one slice) (Sandhu *et al*., 2001). In the other meta-analysis, the investigators reported a 24% increase in risk associated with each daily 120 g increment of red meat intake, and a 36% increase in risk for each 30 g increment of processed meat (Norat *et al*., 2002).

The increased risk of colon cancer associated with red and processed meats has generated several hypotheses related to mutagenic heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) (formed during cooking at high temperatures or over an open flame; Sugimura, 1985). However, quantifying HCA exposure with precision requires detailed information on the type of meat, cooking method, place of preparation, degree of doneness, and external browning; human studies with more detailed measures of exposure are needed (Sinha and Rothman, 1997). Mutagenic N-nitroso (NOC) compounds, formed endogenously from nitrogenous residues from red meat (Bingham *et al*., 2002), and NOC precursors in tobacco and food (Mirvish *et al*., 2002), are also hypothesized to explain the risks associated with red meat. A recent intervention study found that heme iron (form of iron in meat), but not ferrous iron, stimulated endogenous NOC production in healthy volunteers (Cross *et al*., 2003).

A recent review of epidemiologic studies of red meat and prostate cancer determined that 15 of 21 studies found over 30% increased risks associated with higher red meat intakes, six of which were statistically significant (Kolonel, 2001). The mechanism is unclear, but may relate to HCA or PAH content, or to effects of meat components on hormone metabolism. The evidence for other cancers, including breast, bladder, and kidney cancer, is less consistent (World Cancer Research Fund & American Institute for Cancer Research, 1997; Missmer *et al*., 2002). Diets high in red meat also tend to be low in vegetables and track with other unhealthy lifestyle behaviors, which may contribute to the observed associations.

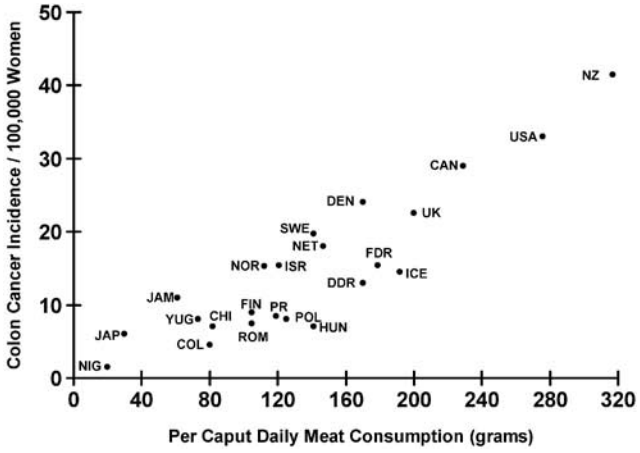

Figure 2 Correlation between colon cancer incidence in various countries and meat consumption (Armstrong and Doll, 1975)

Fruits and vegetables

Fruits and vegetables have long been studied for their role in cancer prevention because they contain numerous substances with potential anticarcinogenic activity, including folate, carotenoids, flavonoids, vitamins, isothiocyanates, dithiolthiones, glucosinolates, allium compounds, and limonene (Steinmetz and Potter, 1991b). Potential mechanisms for cancer prevention include modulation of DNA methylation (Duthie, 1999), prevention of DNA adduct formation (Ames *et al*., 1995), induction of phase II carcinogen-metabolizing enzymes (Lampe, 1999; Lampe *et al*., 2000), alteration of hormone levels (Aldecruetz, 2002), and inhibition of nitrosamine formation and carcinogen-binding (Steinmetz and Potter, 1991b). Results from over 250 epidemiologic studies of fruits and vegetables and cancer have been summarized in several large reviews (Steinmetz and Potter, 1991a; Block *et al*., 1992; World Cancer Research Fund & American Institute for Cancer Research, 1997). In most of these studies, subjects who consume diets high in certain fruits and/or vegetables have lower risk of some, but not all, cancers. However, most of these studies used case–control designs, which are subject to recall bias. Findings from prospective cohort studies of fruits and vegetables in stomach, breast, and colorectal cancer have generated weaker results (World Cancer Research Fund & American Institute for Cancer Research, 1997; Smith-Warner *et al*., 2001b; Terry *et al*., 2001c; IARC, 2003). In addition, an intervention study of fruits and vegetables on recurrence of colorectal adenomas did not find a reduction in risk (Schatzkin *et al*., 2000).

The recent prospective and intervention studies suggest that plant foods may play a smaller direct role in cancer prevention than previously thought. While this may be true, some methodologic aspects of studying fruits and vegetables deserve consideration. It is possible that highest risks occur at very low intakes (Terry *et al*., 2001c), and that most studies exceed this threshold. In addition, many studies combine all fruits and/or vegetables in analyses. As fruits and vegetables contain varying levels of protective compounds, combining them in analyses may obscure potentially strong protective effects of certain phytochemical or botanical subgroups. For example, lycopene-containing (Giovannucci, 1999), cruciferous (World Cancer Research Fund & American Institute for Cancer Research, 1997; Talalay and Fahey, 2001), and allium vegetables (Milner, 2001) are relatively consistently inversely related to the risk of certain cancers. Potatoes and some fruit juices (often included with total vegetables and fruits) might actually increase the risk, as they have a high glycemic index and increase insulin secretion. Potato products also contain high levels of acrylamide, formed during cooking at high temperatures, which has the ability to induce cancer and heritable mutations in animals (FAO/WHO, 2002). In the United States, fruit juice (29%) and potatoes and potato products (27%) contribute importantly to total per capita fruit and vegetable consumption, respectively, while broccoli (0.8%) and dark green vegetables (1%) make up a miniscule amount (Krebs-Smith and Kantor, 2001). Thus, evidence suggests that components of certain fruits and vegetables may protect against specific cancers; however, a blanket protective role for total fruits and vegetables is unlikely.

Dairy

Dairy products are the major source of dietary calcium and vitamin D (discussed below) in the US, and also contain other hypothetically protective (Parodi, 1997) and adverse (Outwater *et al*., 1997) components. Epidemiologic studies of dairy products and colorectal (Platz and Giovannucci, 1999) and breast cancer risk (Missmer *et al*., 2002) have been inconsistent and mostly null. However, recent studies suggest a possible lower risk of these cancers with higher intakes of low-fat dairy products (Shin *et al*., 2002; Wu *et al*., 2002), and a higher risk of breast cancer with high-fat dairy intakes (Cho *et al*., 2003b). Dairy products have been associated in some studies with increased risk of prostate (Chan and Giovannucci, 2001) and ovarian (Cramer *et al*., 2000) cancer. Owing to the potentially beneficial and adverse effects of dairy products, their overall influence on cancer risk remains controversial.

Micronutrients

Vitamin D

Populations with greater exposure to ultraviolet light generally have lower risks of breast (Garland *et al*., 1990), colon (Garland and Garland, 1980), and prostate cancer (Hanchette and Schwartz, 1992). Vitamin D is synthesized in the skin after exposure to UV radiation, and is also obtained in the diet from fortified milk products and breakfast cereals (US), fatty fish, and multivitamin supplements. A protective role for $1,25(OH)_2D$ (the active form of the vitamin) in growth regulation and cell differentiation for each of these cancers has been hypothesized based on experimental data (Giovannucci, 1998; Lipkin and Newmark, 1999). The fact that 25-hydroxyvitamin D (25(OH)D, storage form) can undergo conversion to $1,25(OH)_2D$ in certain colon cancer cell lines (Cross *et al*., 1997), and can inhibit cell growth in prostate cells that express 1-α-hydroxylase (Barreto *et al*., 2000), has important implications for the role of this vitamin in carcinogenesis. However, prostate cancer cells appear to lose 1-α-hydroxylase activity (Hsu, 2001). Low 25(OH)D levels have also been associated with increased colonic epithelial cell proliferation indices (Holt *et al*., 2002), increased adenoma risk (Peters *et al*., 2001), and colorectal (Tangrea *et al*., 1997) cancer. One study reported a 29% reduction in the risk of breast cancer in women with a high dietary vitamin D/sun exposure index compared to a low index (John *et al*., 1999). While epidemiologic studies weakly support a role of vitamin D in reducing risk of these cancers, most studies have generally assessed sunlight, diet or serum levels separately. Future studies will be enhanced if they consider these factors concurrently, in addition to genetic modifiers (discussed below).

Calcium

Dietary calcium has been shown to influence cancer risk in a complex fashion, potentially involving several mechanisms. It has been inversely related to the risk of colorectal cancer (Platz and Giovannucci, 1999) and adenoma recurrence (Baron *et al*., 1999), but high intake of calcium has been positively associated with risk of other cancers, including prostate cancer (Chan and Giovannucci, 2001; Rodriguez *et al*., 2003). Calcium has been hypothesized to protect against colorectal cancer by binding secondary bile acids and ionized fatty acids in the colon to form insoluble soaps, thereby reducing their proliferative stimulus on colonic mucosa (Newmark *et al*., 1984; McMichael and Potter, 1985). Calcium may also directly reduce cellular proliferation in the colonic mucosa or cause terminal differentiation of cells (Lipkin and Newmark, 1985; Bostick, 1997; Lamprecht and Lipkin, 2001).

Although previous epidemiologic studies suggested a weak, if any, relationship with colorectal cancer risk (Martinez and Willett, 1998), recent studies of calcium have shown a more consistent inverse association with colon or colorectal cancer (Wu *et al*., 2002; McCullough *et al*., 2003) or recurrent adenomas (Baron *et al*., 1999; Bonithon-Kopp *et al*., 2000). The optimal dose and form of calcium that may be most protective is not known. Intervention trials of adenomas examined only high supplemental doses of 1200 mg or 2 g per day.

Prospective studies of colon cancer suggest that total calcium intakes above 1000 mg may not confer additional benefit (Wu *et al*., 2002; McCullough *et al*., 2003). The findings for breast and prostate cancer are more equivocal. One hospital-based case–control study (Negri *et al*., 1996) and a recent large prospective study (Shin *et al*., 2002) reported a statistically significant lower risk of breast cancer with high vs low calcium intakes, while three case-control studies did not (Katsouyanni *et al*., 1988; Potischman *et al*., 1999; Levi *et al*., 2001). Case–control studies of calcium and prostate cancer have been inconsistent, but four out of five prospective studies report an increased risk with higher calcium intake (Chan and Giovannucci, 2001; Rodriguez *et al*., 2003), particularly above 1500 mg/day and for advanced (metastatic) prostate cancer (Giovannucci *et al*., 1998a). One hypothesis for this increased risk is that high calcium intakes transiently increase circulating calcium levels, which downregulate production of $1,25(OH)_2D$, the active form of vitamin D. As previously noted, vitamin D reduces cell proliferation and induces cell differentiation *in vitro*. These activities are thought to be mediated by the vitamin D receptor (VDR) (Martinez and Willett, 1998; Holt, 1999). Prostate cells contain VDRs and reduction of $1,25(OH)_2D$ levels in the plasma is thus hypothesized to have adverse effects on carcinogenesis (Giovannucci, 1998).

As colorectal, prostate, and breast cells all contain VDRs, dietary calcium would be predicted to hypothetically increase risk in all of these cancers through reduction of $1,25(OH)_2D$ levels. However, different mechanisms may apply to different cancers. For example, both blood and luminal factors can influence colon cancer (Potter, 1999), and calcium may directly influence cellular proliferation and differentiation (Lipkin and Newmark, 1985). In addition, recent evidence suggests that many tissues have the ability to convert 25(OH)D to 1,25(OH)2D (Cross *et al*., 1997); so in some organs plasma 25(OH)D levels may be more relevant than plasma 1,25(OH)2D levels, which are largely determined by renal 1-α-hydroxylase.

Folate

One of the most important ways that diet may influence carcinogenesis is through effects on DNA synthesis and methylation (Figure 3). Folate is essential for cellular reactions that require the transfer of methyl groups (Giovannucci, 2002). Low folate (in the form of 5-methyltetrahydrofolate) status, whether by inadequate intake or metabolism, reduces intracellular S-adenosylmethionine (SAM), and alters cytosine methylation in DNA, potentially leading to inappropriate activation of proto-oncogenes (Duthie, 1999) or inactivation of tumor-suppressor genes. Further, when levels of 5,10-methylenetetrahydrofolate are low, misincorporation of uracil for thymidine occurs during DNA synthesis (Wickramasinghe, 1994; Blount, 1997), increasing the need for DNA repair, which is also compromised when

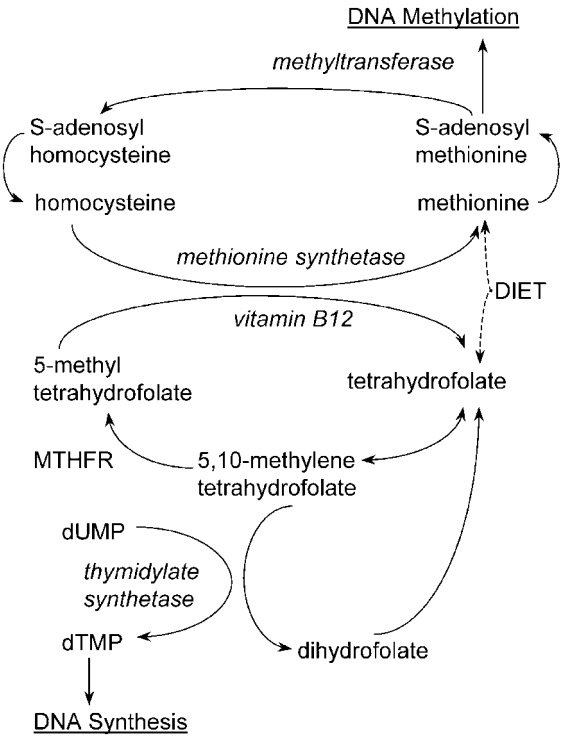

Figure 3 Competing pathways in folate metabolism. MTHFR, 5,10-methylenetetrahydrofolate reductase (Giovannucci, 2002)

folate is limited (Duthie *et al*., 2000). These abnormalities can be reversed with folic acid supplementation.

Epidemiologic studies have linked low folate intake with higher risk of several cancers, most notably colorectal (Giovannucci, 2002), breast and uterine cervix cancer (Eichholzer *et al*., 2001). Dietary folate is obtained from fruits, vegetables, and legumes, and, in the US, folate is available in grains, which have been fortified with this vitamin since 1998 (DHHS, 1996). Long-term use of folate-containing multivitamin supplements has been associated with a 30–75% reduction in risk of colon cancer (Giovannucci *et al*., 1998b; Jacobs *et al*., 2001). Additional dietary cofactors, such as methionine, alcohol, and vitamins B12 and B6 also influence methylation processes. Alcohol interferes with folate absorption and transport, resulting in potent acute and chronic effects on folate metabolism (Hillman and Steinberg, 1982). Animal and human studies have shown that 'methyl poor' diets (high alcohol–low methionine–low folate) are associated with 3–4-fold increases in the risk of both colorectal adenomas and cancer compared to 'methyl-rich' diets (low alcohol–high methionine–high folate) (Giovannucci, 2002). These results are more consistent in men than women, possibly because of the generally higher alcohol intake in men.

Most, but not all, studies report lower risk of breast cancer with higher folate intake or plasma levels (Eichholzer *et al*., 2001; Feigelson *et al*., 2003; Zhang *et al*., 2003). As with colorectal cancer, folate appears to ameliorate the increased risk of breast cancer associated

with alcohol intake in some (Zhang *et al*., 1999; Rohan *et al*., 2000; Sellers *et al*., 2000) but not all (Feigelson *et al*., 2003) studies.

Isolated investigations of other cancers, including esophageal cancer (Prasad *et al*., 1992) and leukemia (Thompson *et al*., 2001), also suggest that inadequate folate intake or metabolism can contribute to carcinogenesis in these sites. Further investigation of folate and other cancers will be necessary to define how broad the effect of a deficiency in this vitamin may be. Interactions of folate and folate cofactors with MTHFR polymorphisms (discussed below) add additional evidence for a causal role of folate in carcinogenesis.

Carotenoids

Carotenoids, partly responsible for the pigment in plants (Olson, 1999), have been examined in relation to cancer risk for many sites. Over 600 carotenoids are present in nature, but only about 20 have been identified in human serum and only five are plentiful (β-carotene, α-carotene, lycopene, lutein/zeaxanthin, β-cryptoxanthin) (Arab *et al*., 2001). The most concentrated sources are fruits and vegetables, and intake levels are reflected in blood and peripheral tissues since they are not synthesized in animals. Carotenoids can act as antioxidants, pro-oxidants, nuclear transcription factors (limited to carotenoids with pro-vitamin A activity), and can reduce tumor formation via IGF-receptor inhibition (Arab *et al*., 2001). The anticarcinogenic potential of β-carotene and lycopene has been most extensively studied.

β-Carotene (and other carotenoids) in diet and prospectively collected sera have been associated with lower risk of lung cancer, but studies of breast cancer have been inconsistent (World Cancer Research Fund & American Institute for Cancer Research, 1997; Toniolo *et al*., 2001). However, intervention studies of β-carotene supplements have been unable to isolate β-carotene as the protective factor vs other components in whole food. Two large chemoprevention trials in male smokers found unexpected increased risks of lung cancer with β-carotene when provided as supplements in doses that far exceed typical dietary intakes (The alpha-tocopherol beta-carotene cancer prevention study group, 1994; Omenn *et al*., 1996). However, β-carotene supplementation did not increase the risk of cancer in intervention studies of mostly nonsmoking men (Hennekens *et al*., 1996) and women (Lee *et al*., 1999). As cigarette smoke can oxidize β-carotene *in vitro*, and oxidized breakdown products of β-carotene may increase cytochrome *P*450 catabolism of retinoic acid (with loss of repression of metaplasia) (Liu *et al*., 2003), the interaction of β-carotene with cigarette smoking has been examined in humans. β-Carotene supplements were shown to reduce the risk of recurrent colorectal adenomas by 44% in subjects who did not smoke or drink, but doubled the risk among those who smoked and consumed more than one drink per day (Baron *et al*., 2003). More work is needed to understand the mechanism for an interaction of tobacco smoke, alcohol, and β-carotene on disease risk in humans. A recent analysis from the Alpha-Tocopherol, Beta-Carotene (ATBC) intervention study of smokers reported that baseline dietary intakes of several carotenoids, as well as baseline serum β-carotene and serum retinal, were all inversely associated with lung cancer risk over 14 years' follow-up (Holick *et al*., 2002). This finding suggests that the form, dose, and timing of carotenoid exposure may also be relevant.

Lycopene from food is relatively consistently related to an inverse risk of some cancers, but no long-term trials of lycopene supplementation have been conducted. Lycopene, the most abundant carotenoid in the plasma in some populations (Olson, 1999), is not converted to vitamin A and thus may be entirely available for other functions, such as anti-oxidation (Giovannucci, 1999). Lycopene is the most effective quencher of singlet oxygen and free radicals among common carotenoids *in vitro* (Woodall *et al*., 1997), and is concentrated in tomatoes and tomato products, watermelon, and pink grapefruit (Arab *et al*., 2001). In a recent review, 57 of 72 epidemiologic studies reported inverse associations between tomato intake or blood lycopene level and the risk of specific cancers, with approximately half reporting at least 40% lower risks (Giovannucci, 1999). In 35 of these studies, the associations were statistically significant, the evidence being strongest for cancers of the prostate, lung, and stomach. However, almost all of the lycopene in these populations comes from tomato sources, so other components in tomatoes may also explain these findings. A role for lutein and zeaxanthin in cancer prevention is possible, but again, whether these specific carotenoids act independently of other beneficial components in fruits and vegetables is still unknown (Mares-Perlman *et al*., 2002).

Antioxidants

Various antioxidants are likely to influence cancer risk in a complex manner. Oxidant by-products of normal metabolism and external factors such as tobacco smoking damage DNA, protein, and lipids; DNA repair enzymes can efficiently repair damage but are imperfect (Ames *et al*., 1995). Antioxidants may reduce the risk of cancer by neutralizing reactive oxygen species or free radicals that can damage DNA. Vitamin C is the major water-soluble antioxidant, and vitamin E is the major lipid-soluble membrane-localized antioxidant in humans. Other plant food constituents including carotenoids and flavonoids have antioxidant activity (in addition to other anticarcinogenic properties) (Yang *et al*., 2001). Various antioxidants probably do not have similar effects throughout the body because each has unique characteristics and distribution within the body (Ames *et al*., 1995).

Epidemiologic studies of dietary vitamin C support an inverse association, particularly for cancers of the stomach, mouth, esophagus, lung, pancreas, and uterine cervix (World Cancer Research Fund & American Institute for Cancer Research, 1997). Vitamin C can interfere with formation of nitrosamines in the stomach, carcinogens formed endogenously from precursors

present in the diet and tobacco smoke. Interaction with *Helicobacter pylori* in stomach cancer has also been suggested to be important (Jacob, 1999). However, chemoprevention trials of stomach cancer in high-risk populations have not conclusively supported a benefit from vitamin C supplements (Blot *et al*., 1993), although several antioxidant nutrients were associated with regression of gastric dysplasia (Correa *et al*., 2000). Vitamin C also interacts with iron to promote oxidative damage (Jacob, 1999). Thus, although some benefit from vitamin C is possible, physiologic effects may be mixed.

Vitamin E (tocopherol) may help prevent cancer by reducing free-radical damage to DNA and through effects on the immune system. Fats and oils are the major sources of vitamin E and the amount and form varies by type of oil (Institute of Medicine, 2000). As the type of oil in the food supply may vary unpredictably, measurement of dietary vitamin E is difficult. In the large ATBC trial, which was restricted to heavy smokers (The alpha-tocopherol beta-carotene cancer prevention study group, 1994), no association between supplemental α-tocopherol and lung cancer was found, but a lower incidence of prostate cancer was observed. Subsequent prospective analyses of vitamin E supplements (usually as α-tocopherol) in prostate cancer support a possible role limited to smokers (Gann *et al*., 1999), with no benefit or even a possible increased risk in non-smokers (Chan *et al*., 1999). Gamma-tocopherol is superior to α-tocopherol in trapping electrophilic species, such as nitrogen oxides or NO(X) (Christen *et al*., 1997). Recently, a striking reduction in risk of prostate cancer was associated with higher levels of serum γ-tocopherol (Helzlsouer *et al*., 2000). Whether supplements (mainly α-tocopherol) contribute to an imbalance of α- to γ-tocopherol ratio and influence prostate cancer risk through a displacement effect is unknown (Christen *et al*., 1997; Giovannucci, 2000). Intervention studies of vitamin E and cancers of the breast and colon (Institute of Medicine, 2000) have not found an appreciable benefit.

Most epidemiologic evidence on the potential anticarcinogenic role of selenium supports a role in reducing cancer risk. Selenium functions through selenoproteins, including selenium-dependent glutathione peroxidases that defend against oxidative stress. The selenium content of food varies more than 10-fold, depending on the selenium content of soil where plants are grown or animals are raised; thus, nutrient databases for selenium are unreliable (Institute of Medicine, 2000). Thus, studies typically test selenium hypotheses not by analysing dietary intake but by utilizing selenium supplements or biochemical markers of intake. Selenium was strongly associated with reduced prostate, lung, and colorectal cancer risk in one trial of selenium supplementation and skin cancer (Clark *et al*., 1996). Two nested case–control studies of toenail and serum selenium biomarkers also reported reduction in risk of prostate cancer (Yoshizawa *et al*., 1998; Nomura *et al*., 2000). The SELECT trial, an ongoing NCI-funded supplement trial, is comparing supplemental selenium, vitamin E (as α-tocopherol), and a combination, compared to placebo, on primary prevention of prostate cancer.

As effects of numerous antioxidants may be additive or interactive, the use of total antioxidant capacity as a biomarker of antioxidant exposure or status has been suggested; however, available methods to measure total antioxidant capacity in plasma or serum or in diet vary considerably (Prior and Cao, 1999). Antioxidant status in the blood is influenced by many factors, however, and unless it is strongly correlated with dietary antioxidant, fruit, or vegetable intake, findings will not be informative on the influence of diet. One study recently applied this technology to foods and reported an inverse association between antioxidant potential of diet (measured via the TRAP assay) and risk of gastric cancer (Serafini *et al*., 2002).

Fiber

Burkitt originally hypothesized that fiber may be responsible for the lower rates of colon cancer in African men, compared to men living in developed countries (Burkitt, 1971). A 1992 meta-analysis of case–control studies supported this hypothesis (Howe *et al*., 1992), but a later re-analysis of these data, considering study heterogeneity and limited to studies with validated diet assessment instruments, did not support a role for fiber (Friedenreich *et al*., 1994). Most prospective cohort studies of dietary fiber and colon cancer risk have not supported an association (Heilbrun *et al*., 1985; Willett *et al*., 1990; Giovannucci *et al*., 1994; Steinmetz *et al*., 1994; Kato *et al*., 1997; Fuchs *et al*., 1999; Pietinen *et al*., 1999; Terry *et al*., 2001a; McCullough *et al*., 2003). However, a recent large European study involving 10 countries found a 25% lower risk of colon cancer associated with higher fiber intakes compared to low intakes (Bingham *et al*., 2003a). This finding may have been due to broader ranges and sources of fiber studied compared to those within individual populations. However, because other potential confounders were not included in the model (e.g. physical activity, folate), residual confounding may have existed. Intervention trials of wheat bran fiber (Alberts *et al*., 2000), isphaghula husk (psyllium fiber) (Bonithon-Kopp *et al*., 2000), and a high-fiber/low-fat diet (Schatzkin *et al*., 2000) failed to reduce the risk of recurrent adenomatous polyps. A prospective study of fiber and breast cancer also did not support an association (Terry *et al*., 2002a).

These apparently contradictory findings among various studies and study designs have additional plausible explanations. Dietary fiber represents several nondigestible food components with different physiologic effects (Goodlad, 2001), possibly with varying effects on carcinogenesis (Ferguson *et al*., 2001), so intervention studies of an isolated purified fiber source do not represent a test of 'dietary fiber'. Plant foods also contain many other potentially anticarcinogenic compounds, so attributing an effect to one dietary component is difficult. Probably the single most challenging aspect of nutritional epidemiology is that nutrients tend

to be highly correlated with one another. For example, dietary fiber is positively correlated with other nutrients in a high-fiber diet (e.g. folate), and inversely correlated with nutrients in a low-fiber diet (e.g. saturated fat). Thus, differences in the analytic approach may also account for conflicting findings in studies. Some investigators have controlled for other potential dietary confounders in high-fiber diets, while others have not. The rationale for doing so, or not doing so, can be debated. The nutrient under study can be hypothesized to explain the entire association with disease risk (only controlling for non-dietary factors), but this is not confirmatory. Burkitt's original hypothesis also emphasized that refined grains and sugars were deleterious (Burkitt, 1971). Possibly, fiber alone is not a strong protective element, but a diet high in refined carbohydrates and low in fiber-rich foods may be deleterious through metabolic effects (e.g. hyperinsulinemia).

Diet patterns

As diets reflect a complex combination of several nutrients and non-nutrients that may have additive or interactive effects on health, investigators have recently attempted to quantify diet patterns in epidemiologic analyses. Several approaches have been used, including creation of diet scores, empirical methods, and intervention feeding studies in humans.

The risk of cancer among individuals who closely follow several dietary guidelines (or theoretically healthy patterns) has been compared to those not following guidelines by the use of multi-dimensional diet scores (Kant *et al*., 1995, 2000; Huijbregts *et al*., 1997; McCullough *et al*., 2000a, 2000b, 2002; Fitzgerald *et al*., 2002; Harnack *et al*., 2002; Michels and Wolk, 2002; Seymour *et al*., 2003; Trichopoulou *et al*., 2003). As most scores have been based on dietary patterns recommended to reduce overall chronic disease risk, they have tended to better predict the risk of cardiovascular disease than cancer. A recent study tested whether an alternative diet pattern to the USDA's Food Guide Pyramid would better predict overall major chronic disease (cardiovascular disease, cancer or other causes of death) in US men and women (McCullough *et al*., 2002). Individuals whose diet was high in fruits and vegetables (excluding potatoes), nuts and soy, cereal fiber, a higher fish and chicken to red meat ratio, a higher polyunsaturated to saturated fat ratio, and low intake of *trans* fatty acids received a higher dietary score. Regular multivitamin use and moderate alcohol consumption also contributed to higher scores. The index predicted nearly twice the reduction in chronic disease risk compared to the USDA Pyramid. Most of the risk reduction was related to lower risk of cardiovascular disease, especially among men. Higher dietary scores did not predict lower incident cancer risk, even after excluding alcohol from the score (McCullough *et al*., 2002). Several factors may have contributed to the weak findings for cancer, including the high proportion of breast and prostate cancers in the study populations, for which newer hypotheses (e.g. lycopene) were not included.

Another approach to studying diet patterns involves identifying correlated eating behaviors from existing data sets (e.g. factor analysis). In some studies, a 'prudent' diet pattern (high in fish, poultry, fruits, and vegetables) has predicted a lower risk of colon cancer (Slattery *et al*., 1998; Fung *et al*., 2003) and a 'Western' diet pattern (high red and processed meat, refined grains, sugars, potatoes) has predicted increased risk, but similar patterns were not as strongly related to colorectal cancer risk in another study (Terry *et al*., 2001b), or breast cancer risk in Swedish women (Terry *et al*., 2001c). Empirical methods have been criticized because of their *post hoc* interpretation, and because results are limited to the specific populations studied. However, they are useful for generating hypotheses. For example, the Western dietary pattern is associated with higher insulin levels (Fung *et al*., 2001), supporting the hyperinsulinemia–cancer hypothesis. Recently, 49% of Western cancers and 29% of all cancers were attributable to a combination of body mass, physical activity, Western dietary pattern, and height (as a proxy of early IGF exposure) (Giovannucci *et al*., 2004). These cumulative findings begin to approach the estimates for diet-related factors and cancer originally proposed by Doll and Peto (1981).

Intervention studies of diet patterns are labor-intensive and costly. Blinding participants to the intervention is often impossible. However, the randomized design and ability to design a specific diet pattern is attractive. A diet high in fiber (high in fruits, vegetables, and whole grains) and low in fat did not lower the risk of recurrent adenomas over a 3–4-year period, compared to standard care (Schatzkin *et al*., 2000). The risk of incident cancer was evaluated in 605 participants with coronary heart disease from the Lyon Diet Heart Study, a study on the effect of a Mediterranean-style diet pattern high in fruits, vegetables, and omega-3 fatty acids on the risk of recurrent myocardial infarction. The investigators found a suggestively lower rate of cancer incidence in the intervention group, but the study was not adequately powered to study this association (de Lorgeril *et al*., 1998). Although the study of diet patterns does not usually allow conclusions to be drawn about the role of individual dietary factors, the evaluation of whole diets, as naturally consumed, is relevant and realistic.

Gene–diet interactions

Common, low-penetrance genotypes can modify the influence of diet on carcinogenesis (or *vice versa*) in experimental studies, potentially with modest individual effects but large public health impact (Vineis, 2001). However, most studies to date have been small, making it difficult to reliably estimate the contribution of specific gene–diet interactions to cancer development.

Most research has focused on the modification of risk associated with cancer susceptibility genes (IARC, 1999) and enhancement or reduction of epigenetic events that influence genetic expression (Duthie, 1999; Ross, 2003).

The influence of diet on susceptibility genotypes has been studied most extensively for the polymorphic xenobiotic-metabolizing enzymes glutathione-*S*-transferase (GST) and *N*-acetyl-transferase (NAT) (Brockton *et al*., 2000; Cotton *et al*., 2000; Hein *et al*., 2000). The glutathione-*S*-transferase M1 (*GSTM1*) and glutathione-*S*-transferase T1 (*GSTT1*) genes code for the cytosolic enzymes GST-μ and GST-θ, which catalyse reactions between glutathione and electrophilic compounds in phase 2 metabolism of xenobiotics (Hayes and Pulford, 1995). In particular, these enzymes detoxify carcinogenic PAHs present in diet (e.g. cooked meat) and tobacco smoke as well as isothiocyanates (mostly in vegetables), which are potent inducers of detoxification enzymes (Lampe and Peterson, 2002). Individuals with the *GSTM1* and *GSTT1* null genotypes are hypothesized to have little or no conjugation activity. Contrary to expectation, individuals who consumed more red meat and who were *GSTM1* or *GSTT1*-null tended to be at lower or similar risk of colorectal cancer compared with men not homozygous for *GSTM1* null who consumed less meat (Gertig *et al*., 1998; Kampman *et al*., 1999). However, a high consumption of cruciferous vegetables rich in isothiocyanates was associated with lower risk of adenomas among those with *GSTM1* and *GSTT1*-null genotypes (compared to low intakes among those with null genotypes), possibly because more isothiocyanates remain in circulation and induce other Phase II enzymes (Lin, 1998).

Two genes, *NAT1* and *NAT2,* code for NATs. *NAT1* and *NAT2* genotypes representing fast acetylators are thought to produce higher levels of reactive compounds that may accelerate DNA damage. In some studies, consumption of well-done meat increased the risk of colorectal cancer markedly among fast-acetylator genotypes (Chen *et al*., 1998; Kampman *et al*., 1999; Brockton *et al*., 2000) especially among ever-smokers (Le Marchand *et al*., 2001), though not all studies have observed an association (Brockton *et al*., 2000; Tiemersma *et al*., 2002; Barrett *et al*., 2003). Results are also suggestive, but inconsistent, for breast cancer: two case–control studies nested within prospective cohorts found elevated risk with greater consumption of red and well-done meat among individuals with fast-acetylator genotypes (Zheng *et al*., 1999; Deitz *et al*., 2000), whereas two case–control studies did not observe an association (Ambrosone *et al*., 1998; Delfino *et al*., 2000).

Currently promising for nutrient–gene interactions is the area of folate-metabolizing enzymes, which are likely to influence DNA methylation patterns, chromosomal stability, and DNA synthesis and repair. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme responsible for catalysing the irreversible reduction of 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate (Figure 3), the primary methyl donor for remethylation of homocysteine to methionine. MTHFR is upregulated when methionine is unavailable from external sources. A common polymorphism of the *MTHFR* gene (677C→T; alanine-to-valine) correlates with reduced enzyme activity (Frosst *et al*., 1995). The TT genotype confers lower risk for colorectal neoplasia if folate status is adequate (e.g. high-folate, high-methionine, low-alcohol intake). The protection may occur because, among TT individuals, the MTHFR enzyme is less efficient at converting 5,10-methylenetrahydrofolate to 5-methyltetrahydrofolate, thus maintaining levels of 5,10-methylenetetrahydrofolate, a cofactor for *de novo* synthesis of nucleotides for DNA synthesis (Chen *et al*., 1996), while folate status is preserved for normal methylation. However, if folate status is poor, TT homozygotes may be susceptible to DNA methylation abnormalities, which may increase the risk of colorectal cancer through an independent pathway. A protective role for the TT genotype under conditions of normal folate status underscores the potential heterogeneous responses to diet by genotype. For breast cancer, three of four studies found that the 677C→T *MTHFR* allele was associated with increased risk (Gershoni-Baruch *et al*., 2000; Campbell *et al*., 2002; Sharp *et al*., 2002; Semenza *et al*., 2003), especially when diet is inadequate in folate (Sharp *et al*., 2002). As several other polymorphic metabolizing enzymes are involved in one-carbon metabolism, a comprehensive analysis of high-risk and low-risk genotype patterns may provide further clues.

Several polymorphisms in the VDR, which functions as a nuclear gene transcription-regulating factor, have been studied in relation to cancer. One example includes *Bsm*I restriction site polymorphisms, which occur in the intron separating exons VIII and IX, and are linked with another polymorphism, a poly(A) microsatellite located in the 3′ UTR (Ingles *et al*., 2000). The BB genotype has been associated with higher circulating $1,25(OH)_2D$ levels (Ma *et al*., 1998), and with lower risk of breast cancer in some (Yamagata *et al*., 1997; Ruggiero *et al*., 1998; Bretherton-Watt *et al*., 2001) but not all (Ingles *et al*., 2000; Hou *et al*., 2002) studies. Findings for colon cancer (Slattery *et al*., 2001) and prostate cancer (Ingles *et al*., 1997) also support a potential protective effect of this polymorphism or haplotypes containing this polymorphism. Interestingly, this genotype is associated with increased risk of osteoporosis (Zmuda *et al*., 2000), possibly because $1,25(OH)_2D$ causes calcium resorption from the bone to restore serum levels. This role of the *Bsm*I allele is consistent with the antagonistic pleiotropic theory of aging (Martin and Martin, 1997), such that polymorphisms associated with higher bone density for reproduction are maintained in the genome, at the expense of higher cancer risk. Interestingly, in recent studies, higher bone mass has been associated with increased risk of prostate and breast cancers (Zmuda *et al*., 2001; Zhang *et al*., 2002). Although dietary factors including calcium and vitamin D influence circulating levels of vitamin D metabolites, little is known about their potential to influence cancer risk according to VDR genotype.

Studies of gene–diet interactions do more than enhance our understanding of heterogeneous responses to an exposure, they also help provide causal evidence for the role of a particular nutrient. For example, consistent findings of interactions between folate status and MTHFR genotypes strengthen the plausibility that folate is important in carcinogenesis.

Summary

The evidence for a role of diet in cancer prevention is continually evolving as new studies accumulate. Table 1 provides a summary of nutritional factors and their relation to risk of major cancers based on current data. The factors listed would undoubtedly vary if summarized by other authors, but the evolving and expanding list does demonstrate substantial progress since Doll and Peto's (1981) report. For example, leading hypotheses even 10 years ago centered on total calories, dietary fat, specific factors in fruits and vegetables (including β-carotene and dietary fiber), and vitamins A, E, and C (Doll, 1992). These hypotheses have been tested, extended, and clarified, and some have been refuted. A role for positive energy balance and obesity in carcinogenesis has been strengthened, while a specific role of dietary fat has weakened. Fruits and vegetables still appear to be protective for several cancers, but as a whole are weaker determinants than originally thought. Specific phytochemicals (e.g. folate, lycopene, flavonoids, fiber) continue to be actively studied, although distinguishing protective elements in plant foods is a daunting task. We appreciate better that supplemental nutrients may have different health effects than nutrients in food (which may be due to dose, form, or presence of other nutrients in food), and that lifestyle behaviors such as smoking can modify risks. Intervention studies have ruled out a specific protective effect against cancer among smokers for β-carotene, and against a role of certain isolated dietary fibers in reducing recurrent adenomas. However, this research has opened new doors for understanding the complex physiological action of isolated supplements in health and disease. Conversely, calcium supplements did confer modest reduction of adenoma recurrence in two polyp prevention trials. Early studies of gene–diet interactions suggest an important potential for public health impact, especially for folate-metabolizing enzymes, but have generally been inconsistent and too small to examine the multitude of potential interactions in complex biological pathways.

Future directions

Many lines of evidence show that nutrients and nonnutrients in the diet have the potential to influence cancer development, but much work remains in identifying specific factors. Future diet studies with

Table 1 Relationship of dietary factors with risk of major cancers[a]

Diet	*Colorectal*	*Breast*	*Prostate*	*Lung*	*Stomach*	*Esophageal*	*Oral*	*Pancreatic*	*Bladder*	*Kidney*	*Endometrial*
Macronutrients/energy balance											
Obesity	↑↑	↑↑			↑[b]	↑[b]		↑		↑	↑↑
GI/GL[c], IGF, height or metabolic syndrome	↑↑	↑	↑					↑		↑	↑
Animal fat			↑								↑
Foods											
Red or processed meat	↑		↑								
Fruits[d]	↓		↓	↓	↓	↓	↓				↓
Vegetables[d]	↓	↓	↓		↓	↓	↓		↓		↓
Nutrients											
Folic acid	↓↓	↓		↓							
Alcohol	↑↑	↑↑				↑↑	↑				
Calcium	↓		↑								
Vitamin D	↓		↓								
β-Carotene *supplements*				↑↑[e]	↓						
Lycopene-containing *foods*			↓	↓	↓						
Vitamin C				↓							
Vitamin E			↓								
Selenium			↓	↓							
Other											
Grilling meat	↑	↑									
Western diet pattern	↑										
High fiber diet	↓										
Salt, preserved foods					↑						
Hot beverages						↑	↑				

[a]Two arrows indicate more consistent evidence. [b]Cancers of the gastric cardia. [c]GI = glycemic index; GL = glycemic load. [d]Evidence for a potential benefit from some components of fruits and vegetables (not necessarily blanket effect). [e]Increased risk limited to smokers

information on genotype will define whether targeted intervention is necessary, and will help establish or exclude causality for nutritional factors. The ability to characterize tumors by stage, grade, histological subtype, and molecular subtype will also clarify the role of modifiable factors in cancer prevention. Mechanisms for a role of diet and cancer will continue to be examined by biologically driven hypotheses. For example, our knowledge of the importance of $1,25(OH)_2D$ in carcinogenesis contributed to studies of calcium and vitamin D, and knowledge of DNA methylation led to studies of folate nutrition. Diet patterns (or lifestyle patterns) capture the complexity of human behavior; thus, research that addresses cumulative effects of multiple exposures is needed. Thus, a combination of obesity, inactivity, and diet patterns may influence insulin and IGF metabolism in an integrative fashion. Understanding the role of diet and lifestyle exposures earlier in life on cancer development may also identify key exposure periods for intervention. At the other end of the spectrum, survival from cancer has improved with greater screening and detection, although much more progress is needed in underdeveloped countries and those less economically advantaged. An improved understanding of the influence of diet on survival from cancer, which may differ from the role of diet in primary prevention, has been understudied and is of high priority. Advances in genotyping and biomarker technologies, combined with maturing large studies of diet and biomarkers in humans, will be integral in moving the field of diet and cancer forward.

References

Albanes D, Jones DY, Schatzkin A, Micozzi MS and Taylor PR. (1988). *Cancer Res.*, **48,** 1658–1662.

Alberts DS, Martinez ME, Roe DJ, Guillen-Rodriguez JM, Marshall JR, Van Leeuwen JB, Reid ME, Ritenbaugh C, Vargas PA, Bhattacharyya AB, Earnest DL and Sampliner RE. (2000). *N. Engl. J. Med.*, **342,** 1156–1162.

Aldecruetz H. (2002). *Lancet Oncol.*, **3,** 364–373.

Ambrosone CB, Freudenheim JL, Sinha R, Graham S, Marshall JR, Vena JE, Laughlin R, Nemoto T and Shields PG. (1998). *Int. J. Cancer*, **75,** 825–830.

Ames BN, Gold LS and Willett WC. (1995). *Proc. Natl. Acad. Sci. USA*, **92,** 5258–5265.

Arab L, Steck-Scott S and Bowen P. (2001). *Epidemiol. Rev.*, **23,** 211–230.

Armstrong B and Doll R. (1975). *Int. J. Cancer*, **15,** 617–631.

Augustin LSA, Dal Maso L, La Vecchia C, Parpinel M, Negri E, Vaccarella S, Kendall CWC, Jenkins DJA and Franceschi S. (2001). *Ann. Oncol.*, **12,** 1533–1538.

Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, Rothstein R, Summers RW, Snover DC, Beck GJ, Bond JH and Greenberg ER. (1999). *N. Engl. J. Med.*, **340,** 101–107.

Baron JA, Cole BF, Mott L, Haile R, Grau M, Church TR, Beck GJ and Greenberg ER. (2003). *J. Natl. Cancer Inst.*, **95,** 717–722.

Barreto AM, Schwartz GG, Woodruff R and Cramer SD. (2000). *Cancer Epidemiol. Biomarkers Prev.*, **9,** 265–270.

Barrett JH, Smith G, Waxman R, Gooderham N, Lightfoot T, Garner R, Augustsson K, Wolf CR, Bishop DT and Forman D. (2003). *Carcinogenesis*, **24,** 275–282.

Bingham SA. (1987). *Nutr. Abstr. Rev.*, **57,** 705–743.

Bingham SA, Day NE, Luben R, Ferrari P, Slimani N, Norat T, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez C, Key TJ, Trichopoulou A, Naska A, Vineis P, Rumino R, Krogh V, Bas Bueno-de-Mesquita H, Peeters PHM, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R and Riboli E. (2003a). *Lancet*, **361,** 1496–1501.

Bingham SA, Hughes R and Cross AJ. (2002). *J. Nutr.*, **132,** 3522S–3525S.

Bingham SA, Luben R, Welch A, Wareham N, Khaw KT and Day N. (2003b). *Lancet*, **362,** 212–214.

Block G, Patterson B and Subar A. (1992). *Nutr. Cancer*, **18,** 1–29.

Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY, Yu YLB, Tangrea J, Sun Y, Liu F, Fraumeni JF, Zhang YH and Li B. (1993). *J. Natl. Cancer Inst.*, **85,** 1483–1492.

Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB and Ames BN. (1997). *Proc Natl Acad Sci USA*, **94,** 3290–3295.

Bonithon-Kopp C, Kronborg O, Giocosa A, Rath U and Faivre J. (2000). *Lancet*, **356,** 1300–1306.

Bostick RM. (1997). *Cancer Epidemiol Biomarkers Prev*, **6,** 971–980.

Branca F, Hanley AB, Pool-Zobel B and Verhagen H. (2001). *Br. J. Nutr.*, **85,** S55–S92.

Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N and Colston KW. (2001). *Br. J. Cancer*, **85,** 171–175.

Brockton N, Little J, Sharp L and Cotton SC. (2000). *Am. J. Epidemiol.*, **151,** 846–861.

Burkitt DP. (1971). *Cancer*, **28,** 3–13.

Calle EE, Rodriguez C, Walker-Thurmond K and Thun MJ. (2003). *N. Engl. J. Med.*, **348,** 1625–1638.

Campbell IG, Baxter SW, Eccles DM and Choong DY. (2002). *Breast Cancer Res.*, **4,** R14.

Carroll KK. (1975). *Cancer Res.*, **35,** 3374–3383.

Chan JM and Giovannucci EL. (2001). *Epidemiol Rev.*, **23,** 87–92.

Chan JM, Stampfer MJ, Ma J, Rimm EB, Willett WC and Giovannucci EL. (1999). *Cancer Epidemiol. Biomarkers Prev.*, **8,** 893–899.

Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC and Hunter DJ. (1996). *Cancer Res.*, **56,** 4862–4864.

Chen J, Stampfer MJ, Hough HL, Garcia-Closas M, Willett WC, Hennekens CH, Kelsey KT and Hunter DJ. (1998). *Cancer Res.*, **58,** 3307–3311.

Cho E, Spiegelman D, Hunter DJ, Chen WY, Colditz GA and Willett WC. (2003a). *Cancer Epidemiol. Biomarkers Prev.*, **12,** 1153–1158.

Cho E, Spiegelman D, Hunter DJ, Chen WY, Stampfer MJ, Colditz GA and Willett WC. (2003b). *JNCI Cancer Spectrum*, **95,** 1079–1085.

Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW and Ames BN. (1997). *Proc. Natl. Acad. Sci. USA*, **94,** 3217–3222.

Clark LC, Combs GFJ, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JLJ, Park HK, Sanders BBJ, Smith CL and Taylor JR. (1996). *JAMA*, **276,** 1957–1963.

Clinton SK and Giovannucci E. (1998). *Annu. Rev. Nutr.*, **18,** 413–440.
Correa P, Fontham ETH, Bravo JC, Bravo LE, Ruiz B, Zarama G, Realpe JL, Malcom GT, Li D, Johnson WD and Mera R. (2000). *J. Natl. Cancer Inst.*, **92,** 1881–1888.
Cotton SC, Sharp L, Little J and Brockton N. (2000). *Am. J. Epidemiol.*, **151,** 7–32.
Cramer DW, Greenberg ER, Titus-Ernstoff L, Liberman RF, Welch WR, Li E and Ng WG. (2000). *Cancer Epidemiol. Biomarkers Prev.*, **9,** 95–101.
Crews H, Alink G, Andersen R, Braesco V, Holst B, Maiani G, Ovesen L, Scotter M, Solfrizzo M, van den Berg R, Verhagen H and Williamson G. (2001). *Br. J. Nutr.*, **86,** S5–S35.
Cross AJ, Pollock JRA and Bingham SA. (2003). *Cancer Res.*, **63,** 2358–2360.
Cross HS, Peterlik M, Reddy S and Schuster I. (1997). *J. Steroid Biochem. Mol. Biol.*, **62,** 21–28.
de Lorgeril M, Salen P, Martin JL, Manjaud I, Boucher P and Mamelle N. (1998). *Arch. Intern. Med.*, **158,** 1181–1187.
Deitz AC, Zheng W, Leff MA, Gross M, Wen W-Q, Doll MA, Xiao GH, Folsom AR and Hein DW. (2000). *Cancer Epidemiol. Biomarkers Prev.*, **9,** 905–910.
Delfino RJ, Sinha R, Smith C, West J, White E, Lin HJ, Liao SY, Gim JS, Ma HL, Bulter J and Anton-Culver H. (2000). *Carcinogenesis*, **21,** 607–615.
DHHS (1996). *Fed. Regist.*, **61,** 8781–8807.
Doll R and Peto R. (1981). *J. Natl. Cancer Inst.*, **66,** 1191–1308.
Doll R. (1992). *Cancer Res.*, **52,** 2024s–2029s.
Duthie SJ. (1999). *Br. Med. Bull.*, **55,** 578–592.
Duthie SJ, Narayanan S, Blum S, Pirie L and Brand GM. (2000). *Nutr. Cancer*, **37,** 245–251.
Eichholzer M, Luthy J, Moser U and Fowler B. (2001). *Swiss Med. Wkly.*, **131,** 539–549.
Enns CW, Goldman JD and Cook A. (1997). *Family Economics and Nutrition Review*, **10,** 2–15.
FAO/WHO (2002). *Health Implications of Acrylamide in Food.* World Health Organization: Geneva.
Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ and Calle EE. (2003). *Cancer Epidemiol. Biomarkers Prev.*, **12,** 161–164.
Ferguson LR, Chavan RR and Harris PJ. (2001). *Nutr. Cancer*, **39,** 155–169.
Fitzgerald AL, Dewar RA and Veugelers PJ. (2002). *Nutr. Cancer*, **43,** 127–132.
Friedenreich CM. (2001). *Cancer Epidemiol. Biomarkers Prev.*, **10,** 287–301.
Friedenreich CM, Brant RF and Riboli E. (1994). *Epidemiology*, **5,** 66–79.
Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP and Rozen R. (1995). *Nat. Genet.*, **10,** 111–113.
Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Stampfer MJ, Rosner B, Speizer FE and Willett WC. (1999). *N. Engl. J. Med.*, **340,** 169–176.
Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, Colditz GA and Willett WC. (2003). *Arch. Intern. Med.*, **163,** 309–314.
Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC and Hu FB. (2001). *Am. J. Clin. Nutr.*, **73,** 61–67.
Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH and Stampfer MJ. (1999). *Cancer Res.*, **59,** 1225–1230.
Garland CF and Garland FC. (1980). *Int. J. Epidemiol.*, **9,** 227–231.
Garland FC, Garland CF, Gorham ED and Young JF. (1990). *Prev. Med.*, **19,** 614–622.
Gershoni-Baruch R, Dagan E, Israeli D, Kasinetz L, Kadouri E and Friedman E. (2000). *Eur. J. Cancer*, **36,** 2313–2316.
Gertig DM, Stampfer M, Haiman C, Hennekens CH, Kelsey K and Hunter DJ. (1998). *Cancer Epidemiol. Biomarkers Prev.*, **7,** 1001–1005.
Giovannucci E and Goldin B. (1997). *Am. J. Clin. Nutr.*, **66** (Suppl)**,** 1564S–1571S.
Giovannucci E. (1998). *Cancer Causes Control*, **9,** 567–582.
Giovannucci E. (1999). *J. Natl. Cancer Inst.*, **91,** 317–331.
Giovannucci E. (2000). *J. Natl. Cancer Inst.*, **92,** 1966–1967.
Giovannucci E. (2001). *J. Nutr.*, **131,** 3109S–3120S.
Giovannucci E. (2002). *J. Nutr.*, **132,** 2350S–2355S.
Giovannucci E, Rimm EB, Liu Y and Willett WC. (2004). *Int. J. Epidemiol.*, **33,** 217–225.
Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A and Willett WC. (1994). *Cancer Res.*, **54,** 2390–2397.
Giovannucci E, Rimm EB, Wolk A, Ascherio A, Stampfer MJ, Colditz GA and Willett WC. (1998a). *Cancer Res.*, **58,** 442–447.
Giovannucci E, Stampfer M, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE and Willett W. (1998b). *Ann. Intern. Med.*, **129,** 517–524.
Giovannucci E, Stampfer MJ, Colditz GA, Manson JE, Rosner BA, Longnecker M, Speizer FE and Willett WC. (1993). *Am. J. Epidemiol.*, **137,** 502–511.
Goodlad RA. (2001). *Gut*, **48,** 587–589.
Hanchette CL and Schwartz GG. (1992). *Cancer*, **70,** 2861–2869.
Harnack L, Nicodemus K, Jacobs DR and Folsom AR. (2002). *Am. J. Clin. Nutr.*, **76,** 889–896.
Hayes JD and Pulford DJ. (1995). *Crit. Rev. Biochem. Mol. Biol.*, **30,** 445–600.
Heilbrun LK, Nomura A, Hankin JH and Stemmerman GN. (1985). *Lancet*, **I,** 925.
Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, Devanaboyina U, Nangju NA and Feng Y. (2000). *Cancer Epidemiol. Biomarkers Prev.*, **9,** 29–42.
Helzlsouer KJ, Huang HY, Albert AJ, Hoffman S, Burke A, Norkus EP, Morris JS and Comstock GW. (2000). *J. Natl. Cancer Inst.*, **92,** 2018–2023.
Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano MJ, Ridker PM, Willett W and Peto R. (1996). *N. Engl. J. Med.*, **334,** 1145–1149.
Higginbotham S, Zhang Z, Lee I, Cook NR, Giovannucci E, Buring JE and Liu S. (2004). *J. Natl. Cancer Inst.*, **96,** 229–233.
Hillman R and Steinberg S. (1982). *Annu. Rev. Med.*, **33,** 345–354.
Holick CN, Michaud DS, Stolzenberg-Solomon R, Mayne ST, Pietinen P, Taylor PR, Virtamo J and Albanes D. (2002). *Am. J. Epidemiol.*, **156,** 536–547.
Holt PR. (1999). *J. Am. Coll. Nutr.*, **18,** 379S–391S.
Holt PR, Arber N, Halmos B, Forde K, Kissileff H, McGlynn KA, Moss SF, Fan K, Yang K and Lipkin M. (2002). *Cancer Epidemiol. Biomarkers Prev.*, **11,** 113–119.
Hou M, Tien Y, Lin G, Chen C, Liu C, Lin S and Huang T. (2002). *Breast Cancer Res. Treat.*, **74,** 1–7.
Howe GR, Aronson KJ, Benito E, Castelleto R, Cornee J, Duffy S, Gallagher RP, Iscovich JM, Dengao J, Kaaks R, Kune GA, Kune S, Lee HP, Lee M, Miller AB, Peters RK,

Potter JD, Riboli E, Slattery ML, Trichopoulos D, Tuyns A, Tzonou A, Watson LF, Whittemore AS, Wu Williams AH and Shu Z. (1997). *Cancer Causes Control*, **8,** 215–228.

Howe GR, Benito E, Castelleto R, Cornee J, Esteve J, Gallagher RP, Iscovich JM, Deng-ao J, Kaaks R, Kune GA, Kune S, L'Abbe KA, Lee HP, Lee M, Miller AB, Peters RK, Potter JD, Riboli E, Slattery ML, Trichopoulos D, Tuyns A, Tzonou A, Whittemore AS, Wu-Williams AH and Shu Z. (1992). *J. Natl. Cancer Inst.*, **84,** 1887–1896.

Huijbregts P, Feskens E, Rasanen L, Fidanza F, Nissinen A, Menotti A and Kromhout D. (1997). *BMJ*, **315,** 13–17.

Hursting SD, Lavigne JA, Berrigan D, Perkins SN and Barrett JC. (2003). *Annu. Rev. Med.*, **54,** 131–152.

Ingles SA, Garcia DG, Wang W, Nieters A, Henderson BE, Kolonel LN, Haile RW and Coetzee GA. (2000). *Cancer Causes Control*, **11,** 25–30.

Ingles SA, Ross RK, Yu MC, Irvine RA, La Pera G, Haile RW and Coetzee GA. (1997). *J. Natl. Cancer Inst.*, **89,** 166–170.

Institute of Medicine (2000). *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. National Academy Press: Washington, DC.

IARC (1999). *Metabolic Polymorphisms and Susceptibility to Cancer*. International Agency for Research on Cancer: Lyon.

IARC (2003). *Fruit and Vegetables*. IARC Press: Lyon.

Jacob RA. (1999). *Modern Nutrition in Health and Disease* Shils ME, Olson JA, Shike M and Ross AC (eds). Lippincott Williams & Wilkins: Philadelphia, pp 467–483.

Jacobs EJ, Connell CJ, Patel AV, Chao A, Rodriguez C, Seymour J, McCullough M, Calle EE and Thun MJ. (2001). *Cancer Causes Control*, **12,** 927–934.

John EM, Schwartz GG, Dreon DM and Koo J. (1999). *Cancer Epidemiol. Biomarkers Prev.*, **8,** 399–406.

Jonas CR, McCullough ML, Teras LR, Walker-Thurmond KA, Thun MJ and Calle EE. (2003). *Cancer Epidemiol. Biomarkers Prev.*, **12,** 573–577.

Kampman E, Slattery ML, Bigler J, Leppert M, Samowitz W, Caan BJ and Potter JD. (1999). *Cancer Epidemiol. Biomarkers Prev.*, **8,** 15–24.

Kant AK, Schatzkin A and Ziegler RG. (1995). *J. Am. Coll. Nutr.*, **14,** 233–238.

Kant AK, Schatzkin A, Graubard BI and Schairer C. (2000). *JAMA*, **283,** 2109–2115.

Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE and Riboli E. (1997). *Nutr. Cancer*, **28,** 276–281.

Katsouyanni K, Willett W, Trichopoulos D, Boyle P, Tricopoulou A, Vasilaros S, Papadiamantis J and MacMahon B. (1988). *Cancer*, **61,** 181–185.

Kolonel LN. (2001). *Epidemiol. Rev.*, **23,** 72–81.

Kolonel LN, Nomura AM and Cooney RV. (1999). *J. Natl. Cancer Inst.*, **91,** 414–428.

Krebs-Smith SM and Kantor LS. (2001). *J. Nutr.*, **131,** 487S–501S.

Kushi L and Giovannucci E. (2002). *Am. J. Med.*, **113,** 63S–73S.

Lampe JW and Peterson S. (2002). *J. Nutr.*, **132,** 2991–2994.

Lampe JW. (1999). *Am. J. Clin. Nutr.*, **70,** 475S–490S.

Lampe JW, Chen C, Li S, Prunty J, Grate MT, Meehan DE, Barale KV, Dightman DA, Feng Z and Potter JD. (2000). *Cancer Epidemiol. Biomarkers Prev.*, **9,** 787–793.

Lamprecht SA and Lipkin M. (2001). *Ann. NY Acad. Sci.*, **952,** 73–87.

Le Marchand L, Hankin JH, Wilkens LR, Pierce LM, Franke A, Kolonel LN, Seifried A, Custer LJ, Chang W, Lum-Jones A and Donlon T. (2001). *Cancer Epidemiol. Biomarkers Prev.*, **10,** 1259–1266.

Lee I, Cook NR, Manson JE, Buring JE and Hennekens CH. (1999). *J. Natl. Cancer Inst.*, **91,** 2102–2106.

Levi F, Pasche C, Lucchini F and La Vecchia C. (2001). *Int. J. Cancer*, **91,** 260–263.

Lin HJ, Probst-Hensch NM, Louie AD, Kau IH, Witte JS, Ingles SA, Franke HD, Lee ER and Haile RW. (1998). *Cancer Epidermiol Biomarkers Prev.*, **8,** 647–652.

Lipkin M and Newmark H. (1985). *N. Engl. J. Med.*, **313,** 1381–1384.

Lipkin M and Newmark HL. (1999). *J. Am. Coll. Nutr.*, **18,** 392S–397S.

Liu C, Russell RM and Wang X. (2003). *J. Nutr.*, **133,** 173–179.

Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L, Hennekens CH and Manson JE. (2000). *Am. J. Clin. Nutr.*, **71,** 1455–1461.

Ma J, Stampfer MJ, Gann PH, Hough HL, Giovannucci E, Kelsey KT, Hennekens CH and Hunter DJ. (1998). *Cancer Epidemiol. Biomarkers Prev.*, **7,** 385–390.

Mares-Perlman JA, Millen AE, Ficek TL and Hankinson SE. (2002). *J. Nutr.*, **132,** 518S–524S.

Margetts BM and Nelson M. (1997). *Design Concepts in Nutritional Epidemiology* 2nd edn. Oxford University Press: Oxford.

Martin GM and Martin GR. (1997). *The Biologic Basis of Aging: Implications for Medical Genetics* In: Rimoin DL, Connor JM and Pyeritz RE (Eds) *Principles and Practice of Medical Genetics* Churchill Livingstone: New York, NY, 439–453.

Martinez ME and Willett WC. (1998). *Cancer Epidemiol. Biomarkers Prev.*, **7,** 163–168.

McCullough ML, Feskanich D, Rimm EB, Giovannuci EL, Ascherio A, Variyam JN, Spiegelman D, Stampfer MJ and Willett WC. (2000a). *Am. J. Clin. Nutr.*, **72,** 1223–1231.

McCullough ML, Feskanich D, Stampfer MJ, Rosner BA, Hu FB, Hunter DJ, Variyam JN, Colditz GA and Willett WC. (2000b). *Am. J. Clin. Nutr.*, **72,** 1214–1222.

McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, Spiegelman D, Hunter DJ, Colditz GA and Willett WC. (2002). *Am. J. Clin. Nutr.*, **76,** 1261–1271.

McCullough ML, Robertson AS, Rodriguez C, Jacobs EJ, Chao A, Jonas C, Calle EE, Willett WC and Thun MJ. (2003a). *Cancer Causes Control*, **14,** 1–12.

McCullough ML, Robertson AS, Chao A, Jacobs EJ, Stampfer MJ, Jacobs DR, Diver WR, Calle EE and Thun MJ. (2003b). *Cancer Causes Control*, **14,** 959–970.

McMichael AJ and Potter JD. (1985). *J. Natl. Cancer Inst.*, **75,** 185–191.

Michaud DS, Liu S, Giovannucci E, Willett WC, Colditz GA and Fuchs CS. (2002). *J. Natl. Cancer Inst.*, **94,** 1293–1300.

Michels KB and Wolk A. (2002). *Int. J. Epidemiol.*, **31,** 847–854.

Miller J. (1994). *Am. J. Clin. Nutr.*, **59,** 747S–752S.

Milner JA. (2001). *J. Nutr.*, **131,** 1027S–11031S.

Mirvish SS, Haorah J, Zhou L, Clapper ML, Harrison KL and Povey AC. (2002). *J. Nutr.*, **132,** 3526S–3529S.

Missmer SA, Smith-Warner SA, Spiegelman D, Yaun SS, Adami HO, Beeson WL, vanden Brandt PA, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, Miller AB, Potter JD, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A and Hunter DJ. (2002). *Int. J. Epidemiol.*, **31,** 78–85.

Negri E, La Vecchia C, Franceschi S, D'Avanzo B, Talamini R, Parpinel M, Ferraroni M, Riliberti R, Montella M, Falcini F, Conti E and DeCarli A. (1996). *Int. J. Cancer*, **65,** 140–144.

Newmark HL, Wargovich MJ and Bruce WR. (1984). *J. Natl. Cancer Inst.*, **72,** 1323–1325.
Nomura AMY, Lee J, Stemmermann GN and Combs Jr GF. (2000). *Cancer Epidemiol. Biomarkers Prev.*, **9,** 883–887.
Norat T, Lukanova A, Ferrari P and Riboli E. (2002). *Int. J. Cancer*, **98,** 241–256.
Olson JA. (1999). *Modern Nutrition in Health and Disease* 9th edn. Shils ME, Olson JA, Shike M and Ross AC (eds). Lippincott Williams & Wilkins: Baltimore.
Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S and Hammar S. (1996). *N. Engl. J. Med.*, **334,** 1150–1155.
Outwater JL, Nicholson A and Barnard N. (1997). *Med. Hypotheses*, **48,** 453–461.
Palmer S. (1983). *Cancer Res.*, **43,** 2509S–2514S.
Parodi PW. (1997). *J. Nutr.*, **127,** 1055–1060.
Pereira MA, Jacobs Jr DR, Pins JJ, Raatz SK, Gross MD, Slavin JL and Seaquist ER. (2002). *Am. J. Clin. Nutr.*, **75,** 848–855.
Peters U, McGlynn KA, Chatterjee N, Gunter E, Garcia-Closas M, Rothman N and Sinha R. (2001). *Cancer Epidemiol. Biomarkers Prev.*, **10,** 1267–1274.
Pietinen P, Malila N, Virtanen M, Hartman TJ, Tangrea JA, Albanes D and Virtamo J. (1999). *Cancer Causes Control*, **10,** 387–396.
Platz EA and Giovannucci E. (1999). *Nutritional Oncology* Heber D, Blackburn GL and Go VLW (eds). Academic Press: San Diego, pp 223–252.
Pollak M. (2000). *Eur. J. Cancer*, **36,** 1224–1228.
Pollak M. (2001). *Epidemiol. Rev.*, **23,** 59–66.
Potischman N, Swanson CA, Coates RJ, Gammon MD, Brogan DR, Curtin J and Brinton LA. (1999). *Int. J. Cancer*, **82,** 315–321.
Potter J. (1999). *J. Natl. Cancer Inst.*, **91,** 916–932.
Prasad MP, Krishna TP, Psricha S, Krishnaswamy K and Quereshi MA. (1992). *Nutr. Cancer*, **18,** 85–93.
Prior RL and Cao G. (1999). *Free Radic. Biol. Med.*, **27,** 1173–1181.
Rodriguez C, McCullough ML, Mondul AM, Jacobs EJ, Fakhrabadi-Shokoohi D, Giovannucci EL, Thun MJ and Calle EE. (2003). *Cancer Epidemiol. Biomarkers Prev.*, **12,** 597–603.
Rohan TE, Jain MG, Howe GR and Miller AB. (2000). *J. Natl. Cancer Inst.*, **92,** 266–269.
Ross SA. (2003). *Ann. NY Acad. Sci.*, **983,** 197–207.
Rossouw JE, Finnegan LP, Harlan WR, Pinn VW, Clifford C and McGowan JA. (1995). *J. Am. Med. Womens Assoc.*, **50,** 50–55.
Ruggiero M, Pacini S, Aterini S, Fallai C, Ruggiero C and Pacini P. (1998). *Oncol. Res.*, **10,** 43–46.
Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL and Willett WC. (1997). *JAMA*, **277,** 472–477.
Sandhu MS, Dunger DB and Giovannucci EL. (2002). *J. Natl. Cancer Inst.*, **94,** 972–980.
Sandhu MS, White IR and McPherson K. (2001). *Cancer Epidemiol Biomarkers Prev.*, **10,** 439–446.
Schatzkin A and Gail M. (2002). *Nature Rev.*, **2,** 1–9.
Schatzkin A, Lanza E, Corle D, Lance P, Iber F, Caan B, Shike M, Weissfeld J, Burt R, Cooper MR, Kikendall JW and Cahill J. (2000). *N. Engl. J. Med.*, **342,** 1149–1155.
Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM and Folsom AR. (2000). *Epidemiology*, **12,** 420–428.
Semenza JC, Delfino RJ, Ziogas A and Anton-Culver H. (2003). *Breast Cancer Res. Treat.*, **77,** 217–223.
Serafini M, Bellocco R, Wolk A and Ekstrom AM. (2002). *Gastroenterology*, **123,** 985–991.
Seymour JD, Calle EE, Flagg EW, Coates RJ, Ford ES and Thun MJ. (2003). *Am. J. Epidemiol.*, **157,** 980–988.
Sharp L, Little J, Schoefield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, Baird JO, Haites NE, Heys SD and Grubb DA. (2002). *Cancer Lett.*, **181,** 65–71.
Shin M, Holmes MD, Hankinson SE, Wu K, Colditz GA and Willett WC. (2002). *J. Natl. Cancer Inst.*, **94,** 1301–1311.
Sinha R and Rothman N. (1997). *Mutat. Res.*, **376,** 195–202.
Slattery ML, Benson J, Berry TD, Duncan D, Edwards SL, Caan BJ and Potter JD. (1997). *Cancer Epidemiol. Biomarkers Prev.*, **6,** 677–685.
Slattery ML, Boucher KM, Caan BJ, Potter JD and Ma KN. (1998). *Am. J. Epidemiol.*, **148,** 4–16.
Slattery ML, Yakumo K, Hoffman M and Neuhausen S. (2001). *Cancer Causes Control*, **12,** 359–364.
Smith-Warner SA, Spiegelman D, Adami HO, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, Miller AB, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A and Hunter DJ. (2001a). *Int. J. Cancer*, **92,** 767–774.
Smith-Warner SA, Spiegelman D, Yaun SS, Adami HO, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Miller AB, Potter JD, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A and Hunter DJ. (2001b). *JAMA*, **285,** 769–776.
Steinmetz KA, Kushi LH, Bostick RM, Folsom AR and Potter JD. (1994). *Am. J. Epidemiol.*, **139,** 1–15.
Steinmetz KA and Potter JD. (1991a). *Cancer Causes Control*, **2,** 325–357.
Steinmetz KA and Potter JD. (1991b). *Cancer Causes Control*, **2,** 427–442.
Sugimura T. (1985). *Mutat. Res.*, **150,** 33–41.
Talalay P and Fahey JW. (2001). *J. Nutr.*, **131,** 3027S–3033S.
Tangrea J, Helzlsouer K, Pietinen P, Taylor P, Hollis B, Virtamo J and Albanes D. (1997). *Cancer Causes Control*, **8,** 615–625.
Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L and Wolk A. (2001a). *J. Natl. Cancer Inst.*, **93,** 525–533.
Terry P, Hu FB, Hansen H and Wolk A. (2001b). *Am. J. Epidemiol.*, **154,** 1143–1149.
Terry P, Jain M, Miller AB, Howe GR and Rohan TE. (2002a). *Cancer Epidemiol. Biomarkers Prev.*, **11,** 1507–1508.
Terry P, Suzuki R, Hu FB and Wolk A. (2001c). *Cancer Epidemiol. Biomarkers Prev.*, **10,** 1281–1285.
Terry P, Terry JB and Wolk A. (2001d). *J. Intern. Med.*, **250,** 280–290.
Terry PD, Jain M, Miller AB, Howe GR and Rohan TE. (2003a). *J. Natl. Cancer Inst.*, **95,** 914–916.
Terry PD, Rohan TE and Wolk A. (2003b). *Am. J. Clin. Nutr.*, **77,** 532–543.
The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group (1994). *N. Engl. J. Med.*, **330,** 1029–1035.
Thompson JR, Gerald PF, Willoughby ML and Armstrong BK. (2001). *Lancet*, **358,** 1935–1940.
Tiemersma EW, Campman E, Bueno de Mesquita HB, Bunschoten A, van Schothorst EM, Kok FJ and Kromhout D. (2002). *Cancer Causes Control*, **13,** 383–393.
Toniolo P, Van Kappel AL, Akhmedkhanov A, Ferrari P, Kato I, Shore RE and Riboli E. (2001). *Am. J. Epidemiol.*, **153,** 1142–1147.
Trichopoulou A, Costacou T, Bamia C and Trichopoulos D. (2003). *N. Engl. J. Med.*, **348,** 2599–2608.

Vineis P. (2001). *Public Health Nutr.*, **4,** 485–491.
Wickramasinghe SN and Fida S. (1994). *Blood*, **83,** 1656–1661.
Willett W and Stampfer MJ. (1986). *Am. J. Epidemiol.*, **124,** 17–27.
Willett WC. (1998). *Nutritional Epidemiol.*, 2nd edn Oxford University Press: New York.
Willett WC. (1999). *Modern Nutrition in Health and Disease* Shils ME, Olson JA, Shike M and Ross AC (eds). Lippincott Williams & Wilkens: Baltimore, pp 1243–1253.
Willett WC, Stampfer MJ, Colditz GA, Rosner BA and Speizer FE. (1990). *N. Engl. J. Med.*, **323,** 1664–1672.
Wolever TMS and Jenkins DJA. (1986). *Am. J. Clin. Nutr.*, **43,** 167–172.
Woodall AA, Lee SW, Weesie RJ, Jackson MJ and Britton G. (1997). *Biochim. Biophys. Acta*, **1336,** 33–42.
World Cancer Research Fund & American Institute for Cancer Research (1997). *Food, Nutrition, and the Prevention of Cancer: A Global Perspective*. American Institute for Cancer Research: Washington, DC.
Wu K, Willett WC, Fuchs CS, Colditz GA and Giovannucci EL. (2002). *J. Natl. Cancer Inst.*, **94,** 437–446.
Yamagata Z, Zhang Y and Asaka A. (1997). *Am. J. Hum. Genet.*, **61,** A388.
Yang CS, Landau JM, Huang MT and Newmark HL. (2001). *Annu. Rev. Nutr.*, **21,** 381–406.
Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB and Giovannucci E. (1998). *J. Natl. Cancer Spectrum*, **90,** 1219–1224.
Yu H and Rohan T. (2000). *J. Natl. Cancer Inst.*, **92,** 1472–1489.
Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, Speizer FE and Willett WC. (1999). *J. Am. Med. Assoc.*, **281,** 1632–1637.
Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA and Hankinson SE. (2003). *J. Natl. Cancer Inst.*, **95,** 373–380.
Zhang Y, Kiel DP, Ellison RC, Schatzkin A, Dorgan JF, Kreger BE, Cupples LA and Felson DT. (2002). *Am. J. Med.*, **113,** 734–739.
Zheng W, Deitz AC, Campbell DR, Wen WQ, Cerhan JR, Sellers TA, Folsom AR and Hein DW. (1999). *Cancer Epidemiol. Biomarkers Prev.*, **8,** 233–239.
Zmuda JM, Cauley JA and Ferrell RE. (2000). *Epidemiol. Rev.*, **22,** 203–217.
Zmuda JM, Cauley JA, Ljung BM, Bauer DC, Cummings SR and Kuller LH. (2001). *J. Natl. Cancer Inst.*, **93,** 930–936.