Nutrition and Mutagenesis

Lynnette R. Ferguson and Martin Philpott

Discipline of Nutrition, Faculty of Medical and Health Sciences, The University of Auckland, New Zealand; email: l.ferguson@auckland.ac.nz

Annu. Rev. Nutr. 2008. 28:313–29

First published online as a Review in Advance on April 9, 2008

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

This article's doi: 10.1146/annurev.nutr.28.061807.155449

0199-9885/08/0821-0313$20.00

Key Words

mutagen, antimutagen, mutation, genomic instability, carcinogenesis, atherosclerosis

Abstract

Diet-related mutagenesis plays an etiologic role in chronic diseases, including cardiovascular disease and cancer. Many dietary mutagens are DNA reactive, leading to distinct spectra of base-pair substitution mutations and structural chromosome changes. Examples include aflatoxin B1, ochratoxin A, ptaquiloside, various pyrrolizidine alkaloids, heterocyclic amines including 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, and polycyclic aromatic hydrocarbons such as benzo[a]pyrene. However, endogenously or exogenously formed reactive species, inhibitors of topoisomerase II enzymes (e.g., flavonoids), of DNA repair (e.g., caffeine), or of the mitotic spindle (possibly acrylamide), also cause mutations, including structural chromosome changes and copy number variants. Genomic instability also results from inadequate nutrient intake (e.g., folate and selenium). Antimutagens include vitamin C, carotenoids, chlorophyllin, dietary fibers, and plant polyphenols acting through various mechanisms. Polymorphisms in genes for nutrient uptake, metabolism, and excretion will affect dietary intake in determining individual risk of disease development. Human studies utilizing nutrigenomic/nutrigenetic technologies will be essential to quantifying and overcoming diet-related mutagenesis.

Contents

NUTRITION AND MUTAGENESIS

A 1981 publication by Doll & Peto identified and attempted to quantify the causes of cancer and was important to the way many people considered the etiology of chronic disease (26). It moved attention away from environmental factors such as pollution or viruses, or occupational factors such as aniline dyes, and turned the focus instead onto dietary factors as a major contributor to disease risk. A recent internationally based report from the American Institute of Cancer Research and World Cancer Research Fund (AICR-WCRF) (1) emphasizes eight key recommendations to reduce cancer through dietary control. In addition to a focus on weight control and exercise, the report suggests eating mostly foods of plant origin while taking care to avoid moldy cereals or other foods. It also recommends limiting the intake of red meats and alcohol and avoiding processed meat. It is of interest that most of these recommendations will reduce the probability of mutagenesis.

Evidence is accumulating for the endogenous formation of mutagens as well as mutagens occurring as dietary components per se. Lifestyle and diet influence the formation of reactive species (RS), a general term that describes reactive oxygen, nitrogen, halogen, or sulfur species (43). Diet may enhance immune response to develop chronic inflammation (37), which indirectly leads to RS. Micronutrient deficiency can be as mutagenic as the addition of mutagens (31). A considerable number of substances can suppress the formation, uptake, metabolism, or consequences of mutagen exposure to counter these effects (37, 38).

This review covers examples of each of these various areas, focusing on proof of uptake and efficacy in humans wherever possible. The authors' intent is to provide a descriptive overview of this important area rather than an in-depth analysis of individual components.

RS: reactive species

SIGNIFICANCE OF MUTAGENESIS IN DISEASE SUSCEPTIBILITY

Dietary mutagens, dietary imbalances, oxidative stress, and chronic inflammation may lead to DNA damage that, if unrepaired, may cause various types of mutations, whose cellular expression may lead to a cellular phenotype characteristic of early or progressively later stages of disease. This is particularly true for chronic degenerative diseases, including cancer, cardiovascular disease, and diabetes (16, 37). Mutagenesis may also accelerate the negative effects of the aging process or enhance diseases that are associated with premature aging, such as Alzheimer's (3). Two of these affected processes are illustrated below.

Carcinogenesis

There is excellent evidence that mutations play a causal role in carcinogenesis. It appears that tumor initiation occurs through mutation of somatic cells, whereas later stages may involve other processes, including rapid cell proliferation, gene amplification, and chromosomal rearrangements. Epigenetic changes, including DNA methylation, histone modifications, and microRNA, are also important, and have been reviewed elsewhere (18, 25, 35).

Figure 1 schematically illustrates the initiation and development of a generalized type of cancer, showing key points of interaction of dietary chemicals. Mutation research has traditionally focused on DNA-reactive carcinogens, which typically interact with DNA following metabolic activation. Halliwell (43) considers the importance of the generation of RS, indirectly damaging the DNA. We point to chronic inflammation, which may be modulated through diet, as a potential alternative source of various types of RS (37). It is also possible to identify a third group of mutagens—those inhibiting topoisomerase II (topo II) enzymes (32), those inhibiting DNA repair, and mitotic spindle inhibitors—as mutagens whose primary effect will be at the chromosome level. These processes may be involved at later stages in the carcinogenesis process. The earliest and possibly best-studied cancer type is colorectal cancer, for which several key laboratories have developed and refined a hypothesis involving the accumulation of multiple and different types of mutation as well as epigenetic changes (14, 18, 30).

Cardiovascular Disease

It appears that at least some human atherosclerotic plaques are of monoclonal origin (11). Bridges et al. (16) suggested that somatic mutations play a key role at an early stage in atherosclerosis, and this hypothesis has been developed further by several other authors, including Ross et al. (69), Andreassi et al. (5), and Nair et al. (58). DNA adducts, oxidative DNA damage (47), and several types of genetic lesions have been demonstrated in human atherosclerotic plaques. These include loss of heterozygosity, microsatellite instability, copy number variants, and structural chromosome changes. The schematic process and possible involvement of dietary mutagens is illustrated in **Figure 2**.

Topo II: topoisomerase II

Bartsch & Nair (10) reviewed the evidence that miscoding etheno-DNA adducts are generated by oxidative stress, increase with time in chronically inflamed target organs, and play a mechanistic role in atherosclerosis. Penn & Snyder (62) suggested that atherosclerotic plaque development is also promoted by dietary exposures to mutagens including polycyclic aromatic hydrocarbons such as benzo(a)pyrene. These not only form bulky DNA adducts, but also lead to oxidative stress through redox cycling of quinone intermediates, resulting in lipid peroxidation (50). 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine are highly miscoding lesions (9) that result from the action of RS on polyunsaturated fatty acids. Curfs et al. (24) suggested that TGFβ-mediated local inflammatory reaction in the vessel wall leads to an increased influx of proinflammatory cells into the plaques, which in turn causes further generation of RS and various types of DNA damage (**Figure 2**). A similarity of causal mechanisms between carcinogenesis and cardiovascular disease means that the risks of both can be reduced by bioactive dietary components and/or dietary modulation (37, 51).

DIETARY MUTAGENS

The *Salmonella*/mammalian microsome mutagenicity test developed by Ames and coworkers incorporated a rodent liver microsomal mix (S9 mix) and utilized a series of histidine auxotrophs that could be reverted by quite specific mutagenic events (4). Although mutagenicity tests had been available for some time, the protocols and *S. typhimurium* strains used in the Ames test had advantages over competing systems. Preliminary reports of a persuasive correlation between mutagenicity and carcinogenicity led to

the use of this correlation and related mutagenicity tests as a tool for discovering potential environmental mutagens as well as a predictive test for carcinogenesis.

Through the 1970s and 1980s, many chemicals from various sources (environment, occupation, diet) were tested for mutagenic effects in the Ames test (2, 74). Ames (2) suggested that natural chemicals, present in the human diet as complex mixtures, may be a more important source of human mutation than environmental or occupational exposures. More recent work, which involves testing in more sophisticated assays, has confirmed this conclusion.

The most effective use of these simple microbial mutation tests has been for high-throughput screening of a wide range of potential dietary mutagens, whether tested alone, in combination with known comutagens, or as part of a complex mixture. Positive results prioritize such materials for more extensive testing using mammalian assays and/or long-term in vivo assays. Many of the materials identified in the text below were originally identified in simple in vitro tests but have been subjected to more intensive and relevant testing.

Mutagens can be present in food items as eaten naturally, through fungal contamination, or through cooking or preserving methods. The following provide a range of relevant examples rather than an exhaustive survey. The relevant chemical structures of the main mutagens discussed are illustrated in **Figure 3**.

In the discussion below, dietary mutagens have been classed in terms of their likely mechanism of action wherever possible.

DNA-Reactive Mutagens

Components of a natural diet. Ptaquiloside: Cows that graze in pastures containing bracken fern (genus *Pteridium*) have a high incidence of urinary-bladder papilloma (28); milk from such cows can cause cancer in rodents. This increased cancer risk has been traced to the presence of the norsesquiterpene glucoside, ptaquiloside. Under certain circumstances, this chemical loses a glucose moiety to form a dienone intermediate that is capable of binding to DNA. It alkylates DNA in the minor groove at the N3 position and in the major groove at N7, stimulating the initiation of mismatch

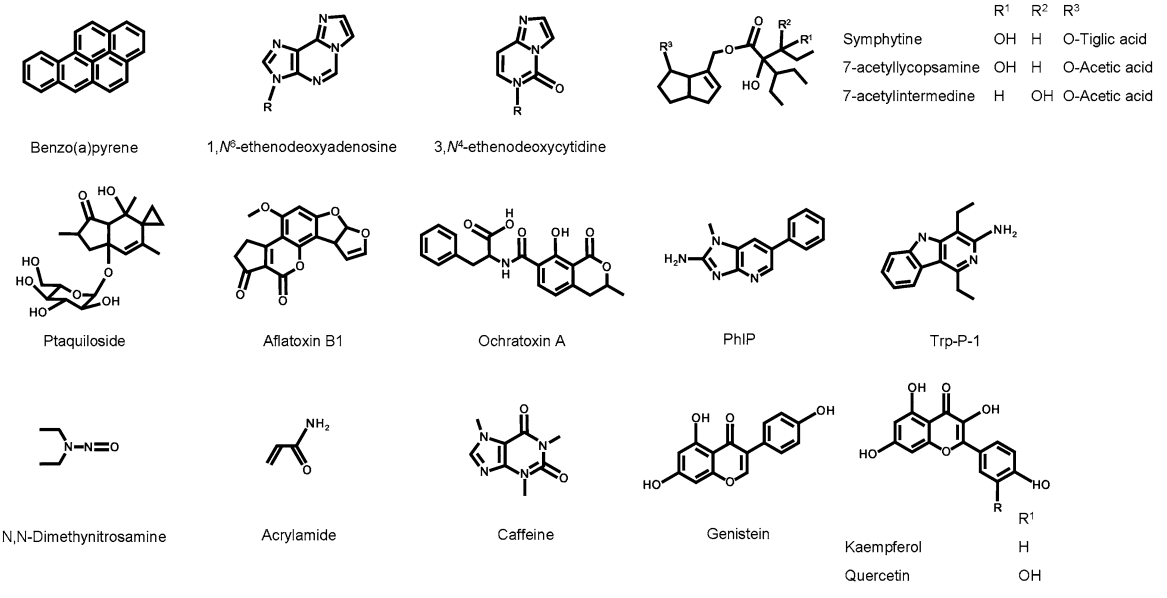

Figure 3

Structures of dietary mutagens (discussed in the text). N,N-dimethylnitrosamine is given as an example of an N-nitroso compound.

repair and mutations in oncogenes including H-*ras* and *neu* (70).

Pyrrolizidine alkaloids (PAs): PAs are common in food plants, especially those used as traditional medicines. Humans may be exposed to this group of compounds through directly ingesting the plants or plant products as herbs, herbal teas, or herbal remedies, or through secondary products such as honey or milk. Fu et al. (40) recently reviewed the properties of chemicals in this field. The parent alkaloid requires dehydrogenation to a pyrrole to become DNA reactive. Of the more than 6000 chemicals commonly found in plants, DNA reactivity (following such activation) and mutagenic properties have been associated with approximately half of the identified structures. However, in vivo data are relatively limited, and the human significance of PAs has been disputed (67). For weak mutagens, standard in vivo mutagenicity testing protocols may be too insensitive to prove definitely effects that are likely to extrapolate to humans. It would be too expensive and, given current standards, unethical to utilize the very large numbers of animals that would be necessary to prove an effect. Transgenic animal models, such as the Big Blue mouse, utilize an in vivo exposure situation and an in vitro mutagenesis assay (55). Cells are harvested from the animal and treated to release a bacteriophage such as the lambda phage cII transgene, whose mutational properties can be tested on a bacterial plate. Large numbers of such plates can be tested, providing the necessary statistical significance for increased (weak) mutagenesis without the ethical dilemmas associated with high numbers of animals. In vitro studies can also be done utilizing this highly sensitive test protocol. Such technologies also enable the characterization of distinctive mutational signatures of various types of mutagen.

Comfrey (*Symphytum officinale*) is used both as a vegetable and herbal remedy by humans. The roots contain high levels of several PAs, including symphytine, 7-acetyllycopsamine, and 7-acetylintermedine. Mei et al. (55) showed that regular consumption of comfrey led to mutagenic effects in the liver of transgenic Big Blue rats. The distinct pattern of mutations may provide a mutational signature for the genetic damage resulting from feeding with plants containing high levels of PAs (55). Such data can be used to correlate animal studies with subsequent effects in humans.

PAs: pyrrolizidine alkaloids

OTA: ochratoxin A

AFB_1: aflatoxin B_1

Mycotoxins. Leblanc et al. (53) used data from the first French Total Diet Study to estimate potential dietary exposure to principal mycotoxins, including aflatoxins, ochratoxin A (OTA), zearalenone, fumonisins, and patulin. They calculated likely points of exposure, depending upon pattern of dietary preferences. Their concerns focused on OTA and zearalenone, where they concluded that cereals and cereal products were likely to be significant contributors to mutagen exposure in certain population groups. Aflatoxin B_1 (AFB_1) has also been shown to be a serious problem in other parts of the world.

Aflatoxin B_1: This metabolite is produced by the fungus *Aspergillus flavus*, which grows on poorly stored foods including corn, peanuts, and rice (17). Those populations with high exposures to this fungal metabolite also have an increased incidence of hepatocellular carcinoma (HCC), especially in association with exposure to hepatitis B virus (46). It is of interest that approximately 50% of HCC samples from people living in such areas show a p53 mutation, characterized by a mutational hot spot in the form of a GC→TA transversion at the third position of codon 249 (46). This may coincide with the observation that the mutational spectrum of cells treated with AFB_1 shows a high preponderance of GC→TA transversions despite the fact that the *exo*-8,9-epoxide formed through metabolic activation of AFB_1 reacts with guanine to form a number of adducts (27). These observations, in combination with other epidemiological and experimental data, suggest a synergistic effect of AFB_1 and HBV on HCC formation (72).

Ochratoxin A: Pfohl-Leszkowicz & Manderville (63) reviewed the toxicology of OTA, a mycotoxin produced through fungal contamination of badly stored food products. Following oxidative metabolism, OTA forms a

PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

Trp-P-1: 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole

DNA-reactive quinone that can form guanine-specific DNA adducts. While these adducts provide an important source of mutation, OTA also forms mutations through the formation of RS. Very potent mutagenic effects can be shown in *S. typhimurium* TA 1535, 1538, and 98, providing certain preincubation protocols and types of metabolic activation are used. Mammalian cells treated with a dose range of this toxin will accumulate single-strand DNA breaks, as revealed by the alkaline single-cell gel electrophoresis (COMET) assay. This effect is greatly enhanced by the addition of an external metabolizing system in the form of S9 mix from rat liver. Again, these effects in vitro correlated with in vivo data (63).

Mutagens formed through cooking or processing. Heterocyclic amines: Early work from Nagao and associates (57) revealed that materials extracted from the surface of charred beef and grilled sardines were mutagenic in the *Salmonella*/mammalian microsome mutagenicity test, after metabolic activation. Commoner and coworkers (21) identified a range of chemicals in extracts from broiled hamburger patties. Mutagens were formed when ground beef was cooked in a home hamburger-cooking appliance or when beef stock was concentrated by boiling to form beef extract. Subsequent work by a considerable number of laboratories has confirmed mutagenic potential in a wide range of systems and extended this to strongly suggest significance for humans. Turesky (75) provides a recent review on the formation and biochemistry of heterocyclic amines.

2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) (**Figure 3**) can be formed during the cooking of muscle foods, especially beef, lamb, and poultry, but also in fish. These and related compounds may also be present in the diet through their presence in environmental contaminants including tobacco smoke and diesel exhaust. Although early observations reported effects in cellular assays or in animal surrogates, the detection of DNA adducts in human tissues suggests that they are likely to be human mutagens. More recent mutagenicity studies have shown in vivo effects with distinctive mutational signatures. For example, Nakai et al. (59) considered effects of PhIP in Fisher 344 (Big Blue) rats. Treatment of rats by this mutagen showed organ-specific effects, causing cancer in the rat ventral prostate but not in the dorsolateral and anterior lobes. These effects failed to relate to direct mutagenesis but did correlate with an increase in the number of stromal mast cells and macrophages that was primarily seen in the ventral prostate. That is, a major source of mutagenicity by heterocyclic amines may be through their causing an inflammatory response rather then directly through adduct formation. This would seem to parallel results from Yun et al. (81), who showed that Trp-P-1 induced nitric oxide production in murine macrophages. Nitric oxide and its oxidized derivatives lead to mutagenesis through an increase in intracellular RS that can directly damage DNA.

Polycyclic aromatic hydrocarbons: Benzo(a)pyrene and related polycyclic aromatic hydrocarbons are known products of incomplete combustion processes (64). Although polycyclic aromatic hydrocarbons may be formed directly in well-cooked meats, fish, etc., they also occur commonly as environmental contaminants on food plants, e.g., cereals and vegetables. Through polluted seawater, they can be accumulated in the tissues of shellfish (36). These compounds are subject to metabolic conversion through the addition of an epoxide group and two OH groups. This metabolic product binds to DNA to form an adduct that is a mutation precursor.

Pavanello et al. (61) considered risk factors for formation of the active species, the antibenzo[a]pyrene diol epoxide-DNA adduct, in lymphocytes in a northeast Italian study population of 585 Caucasian subjects. These were all municipal workers who were recruited during their periodic check-up. Although the work established that environmental factors and

smoking were key risk factors, the study also revealed that diet was a significant source of human exposure to this environmental pollutant. It is likely that such materials not only are generated during some cooking procedures, but also are deposited on the surface of some foods through environmental exposures of food plants.

N-nitroso compounds: These compounds are formed endogenously from nitrates in the body in reaction with amines and have been recognized as mutagens and potential carcinogens since the 1970s (54). Potential precursors can be found in a wide variety of foods, including processed meats such as bacon, ham, and sausage, smoked fish, and certain cheeses. Bingham and associates (13) posed the question of whether increased endogenous formation of N-nitroso compounds in the human colon explains the association between red meat and colon cancer. Their starting hypothesis was partly based on the knowledge that G→A transitions in K-ras, common in colorectal cancer, would be consistent with the mutational spectrum associated with N-nitroso compounds (NOCs). They showed that increased red meat consumption also increased fecal NOC levels in eight male volunteers who consumed diets with extreme (low or high) meat contents. Thus, their work confirmed that an increased endogenous production of NOCs was associated with higher red meat consumption, although this did not establish a causal link with colorectal cancer.

Interest in N-nitroso compounds is likely to be rekindled with the publication of the AICR-WCRF report (1). One of the key recommendations of this large internationally based report on diet, nutrition, physical activity, and cancer is to avoid eating processed meats.

Reactive Species as Mutagens

The chemical nature and formation of RS is described by Wiseman & Halliwell (79) and Halliwell (43) and references therein. RS can cause a range of different DNA lesions, including single- and double-strand DNA breaks, apurinic sites, and modified pyrimidines and purines, subject to repair either through base excision repair or through nucleotide excision repair. Two common events would include direct oxidative damage to DNA resulting in single- or double-strand DNA breaks, or DNA adducts formed through the aldehyde products of lipid peroxidation such as malondialdehyde. It should be noted that individual RS vary in their reactivity, and not all lead to damaged DNA. However, the end point of the DNA reaction can be various types of mutation, including base-pair substitution mutations, deletions or insertions of various sizes, or more gross structural alterations at the chromosome level including chromosomal translocations, as well as gene amplification. RS may also facilitate the conversion of procarcinogens into ultimate carcinogens, some of which then lead to more RS formation. Halliwell (43) describes other mechanisms leading to mutation.

NOC: N-nitroso compound

RS may be formed endogenously as a by-product of oxidative metabolism, through exposure to dietary mutagens, or through pathophysiological states including chronic inflammation. The relationship between inflammation and the production of RS has been extensively reviewed by several authors, including Wiseman & Halliwell (79), Balkwill & Mantovani (7), and Coussens & Werb (23). Invading pathogens, foreign bodies, or infections can lead to inflammation, which is characterized by not only an increased blood supply, but also by the infiltration of white blood cells, including granulocytes, monocytes, and lymphocytes. This in turn leads to the release of soluble mediators including eicosanoids and cytokines that stimulate inflammatory cells (neutrophils, eosinophils, and mononuclear phagocytes). Various stimuli may lead to the activation of oxidant-generating enzymes that release diverse RS. Under certain circumstances, inflammation becomes chronic, leading to a continuous and unbalanced production of RS, which often results in extensive tissue damage as well as DNA damage.

ACR: acrylamide

Inhibitors of Mitotic Spindle, DNA Repair, and Topoisomerase II Enzymes

Although this group of materials is not usually classed together, they show certain similarities in their mutational characteristics. That is, they typically do not cause base-pair substitution mutation but rather are likely to lead to breakage or interchange of chromosomes or to cause gene or chromosomal copy number variants (32, 71).

Acrylamide (ACR) as a mitotic spindle inhibitor: Not only is ACR used in many industries around the world, it also forms naturally in foods cooked at high temperatures. Although it is clearly an animal carcinogen and neurotoxin, extrapolation of effects in cell systems and in animals to effects in humans have been controversial (29). Levels of this compound are high in potato- and cereal-based products subjected to heat processing, such as frying, grilling, or baking. Besaratinia & Pfeifer (12) studied effects of ACR on embryonic fibroblasts from the Big Blue mouse, which carry a lambda phage cII transgene. Micromolar concentrations increased the frequency of mutations in the cII gene up to twofold relative to control treatment, and this was associated with a distinctive mutational spectrum that included an excess of G→C transversions and A→G transitions. However, ACR proved to be mostly negative in other gene mutation assays, other than at high (not dietary) doses. The review by Exon (29) concluded that a major interaction of ACR was with the kinesin proteins that form spindle fibers in the nucleus. This could interfere with accurate chromosome segregation, leading to clastogenicity and aneuploidy. The review also concluded that ACR is likely to interfere with DNA repair, a characteristic of several comutagens and antimutagens.

Caffeine as a DNA repair inhibitor: One of the earliest reports of a suspect human mutagen was in relation to the common dietary constituent, 1,3,7-trimethylxanthine, more commonly known as caffeine. Addition of a dose range of caffeine to the culture medium of either cultured human cells or human leukocytes led to evidence of chromosomal damage and complex interchanges (60). In more than a thousand publications since then, various authors have argued as to the significance of events occurring at such high doses and about whether caffeine is a human mutagen. Porta et al. (66) have reviewed some of this literature. Caffeine can affect cell cycle checkpoints and is an inhibitor of both DNA repair and carcinogen metabolism. This means that it is possible to demonstrate that caffeine is comutagenic, nonmutagenic, and/or antimutagenic, depending upon test system and protocol. Although it occurs naturally at moderate levels in drinks such as tea, caffeine is found at higher levels in coffee and reaches very high levels in some sports or so-called energy drinks.

Genistein as a topoisomerase II inhibitor: The DNA-associated enzyme topo II functions to resolve potential knots and tangles in DNA by inducing transient double-strand breaks. This enzyme has provided a target for an important class of anticancer drugs, and many of the best-studied examples have been developed for this purpose (36). The presence of topo II inhibitors during DNA replication leads to a stabilization of a topo II–DNA cleavable complex and increases the risk of chromosomal breaks and translocations, some in quite specific locations, such as *mixed-lineage leukemia* translocations in primary stem cells. Thus, chemicals that inhibit the action of this enzyme provide an excellent example whereby chromosomal breakage and mutagenicity occur, independent of DNA adduct formation (36).

Flavonoids are one group of the wider class of polyphenols that are widespread in the plant kingdom and many dietary constituents. They are commonly found in fruits and vegetables, cereals, nuts, and beverages (such as beer, red wine, and cocoa). Bandele & Osheroff (8) summarized the evidence for representatives of this class, in the form of flavones, flavonols, and isoflavones, acting as poisons of human topo II alpha and II beta. Genistein was the most active of the compounds they tested, stimulating enzyme-mediated DNA cleavage

approximately tenfold without requiring redox cycling for activity. Flavonoids of this type have been suggested as etiological agents in specific types of childhood leukemia that is characterized by a distinctive chromosomal translocation (68). Although epidemiology has provided some evidence for this hypothesis, it is difficult to be certain of the contribution of one component in a complex mixture. However, the recent demonstration (76) that kaempferol, genistein, and quercetin induce myeloid/lymphoid or mixed-lineage leukemia translocations in primary human stem cells with characteristics of the translocations in this leukemia is entirely consistent with this hypothesis.

MUTAGENESIS THROUGH DIETARY DEFICIENCIES

Mutations may be related to the absence, rather than the presence, of dietary components, especially micronutrients (31). Nutrition recommendations traditionally have been based on prevention of deficiency diseases. The recommended dietary allowances (RDAs), which are updated periodically to reflect new knowledge, state the amount of a nutrient per day that is needed for most people to stay healthy. They are almost certainly too low to maintain genomic stability for all the population. Two examples follow, and others are discussed in some detail by Ames (3).

Folate: Higher intakes of green leafy vegetables have been suggested to reduce the risk of cardiovascular disease and various cancers. This effect has been suggested to relate to the presence of the water-soluble B vitamin, folate, one of the major micronutrients in such vegetables. Methyl group availability appears to be an underlying mechanism for an effect of folate on chronic disease. Courtemanche et al. (22) showed that a deficiency of folate leads to a level of DNA damage comparable to that caused by high-dose radiation.

Selenium: This micronutrient is deficient in some parts of the world, including certain parts of China and New Zealand. We have shown that approximately half of a population group of Auckland men showed serum selenium levels sufficiently low to enhance measurable levels of DNA damage, as assessed using COMET methodology (48). At least in this population, selenium levels would appear to be generally suboptimal. However, it is also important to gauge the correct upper level of such a micronutrient. Waters and associates (78) conducted a randomized feeding trial in which 49 elderly beagle dogs received different levels of dietary selenium over seven months, and then related selenium intake to levels of DNA damage in the prostate. They described a U-shaped dose-response curve for optimal selenium intake to reduce DNA damage. Too much was as damaging as too little.

Ames (3) has suggested that at suboptimal micronutrient intakes, regulatory mechanisms may allocate those micronutrients that are deficient by some sort of triage system in order to optimize current functions. However, this process would reduce the amounts available for maintenance events and thus accelerate the degenerative processes associated with chronic diseases and aging.

RDAs: recommended dietary allowances

DECREASING DIET-RELATED MUTAGENESIS

A large number of in vitro and animal studies have identified a broad range of antimutagens in both natural foods and dietary supplements (for reviews, see 1, 34, 38). An illustrative but far from exhaustive range of examples follows.

Micronutrients: Where nutritional deficiencies are responsible for mutagenesis, the most obvious way to protect against this is to restore the desirable balance of those micronutrients. However, many micronutrients also have other effects that will protect against mutation. These include antioxidant effects (42). For example, vitamin C inhibits the formation of N-nitroso compounds from nitrite and amines (56). (For other examples, see 3 and 31.)

Carotenoids: Although carotenoids are not necessarily micronutrients themselves, representatives of this group are important precursors of vitamin A. Around 40

XME: xenobiotic metabolizing enzymes

different carotenoids are potentially present in the typical human diet. Provitamin A compounds include α-carotene, β-carotene, and β-cryptoxanthin, since they can be converted into retinol, whereas lutein, lycopene, and zeaxanthin have no retinol activity (19). Whether or not they have provitamin A activity, carotenoids have a range of diverse biologic functions that may reduce mutagenic activity of other compounds. Many of them are antioxidants and immunonutrients that can reduce the formation of chromosome aberrations by various dietary mutagens in various model systems. Their human implications have been disputed since the decoding and publication of two major human intervention studies with high-dose β-carotene that appeared to show an increase rather than decrease in both cardiovascular disease and cancer. As Halliwell argues (42), it seems likely that the dose and type of antioxidant selected simply failed to protect against oxidative damage.

Chlorophyll and chlorophyll derivatives: Both natural chlorophyll and more stable derivatives such as sodium copper chlorophyllin appear to have direct antimutagenic effects through binding a range of electrophilic compounds. This leads to an insoluble complex that is excreted from the body before the mutagen in question is able to interact with the cellular material. The cancer-protective effects of such molecules have recently been reviewed by Ferruzzi & Blakeslee (39), who claim that the antimutagenic properties of the molecules relate to anti-inflammatory effects, antioxidant activity, and modulation of xenobiotic metabolizing enzymes (XME).

Dietary fiber: The original concept of dietary fiber was of plant cell walls, and these still comprise most of the dietary fiber in Western diets. However, plant cell walls vary enormously in structure and composition, depending on the species of food plant and types of cells within these (6). Experimental studies have confirmed that different plant cell walls have different properties that are likely to affect disease risk in diverse ways (44). Thus, contrasting food preferences in different population groups may lead to apparently similar dietary-fiber intakes, but these intakes are of very different plant cell walls, with different implications for disease. Several, but not all, of the protective mechanisms for dietary fiber relate to antimutagenesis (44).

Plant polyphenols: This large class of more than 8000 compounds contains many examples of antimutagens acting through various mechanisms, including affecting the metabolic activation of various procarcinogens (31). Various examples have shown powerful antioxidant activities in vitro, through free radical scavenging activity of a wide range of RS, including hydroxyl radicals, peroxyl radicals, hypochlorous acid, superoxide radicals, and $O_2^{\bullet -}$. Flavonoids are one of the major groups of polyphenols and contain several examples that may chelate with and therefore decrease the ability of transition metal ions such as iron and copper to promote RS formation. These properties have been reviewed by Halliwell (42), who argues that the antioxidant potential of this group of compounds may be underestimated because the technologies that have been used thus far have not been sufficiently sensitive.

It is important to realize that not all polyphenols or even all flavonoids are alike, and not all have potentially beneficial properties. Additionally, both mutagenic and antimutagenic properties have been ascribed to several members of this group. An example is provided by the isoflavone genistein. Although the topo II inhibitory properties associated with this chemical might imply that it is an effective mutagen and potential dietary hazard, other studies point to antimutagenic effects. For example, Steiner et al. (73) showed that preincubation of MCF-10A cells with genistein reduced DNA damage caused by two potential dietary mutagens—4-hydroxy-2-nonenal (150 microM) and benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide (50 microM). The mechanism appeared to be through upregulation of glutathione S-transferases, and the authors extrapolated these results to suggest the potential of this mechanism in preventing genotoxic injury in the etiology of breast cancer.

INFLUENCE OF GENOTYPE ON DIETARY MUTAGENESIS

Variants in a wide range of genes will impact the effect of diet to the individual. Two classes of genes have been extensively studied in relation to diet-related mutation.

Xenobiotic Uptake, Metabolism, and Excretion

For many of the mutagens described in the preceding text, the probability that they will be absorbed, reach the target tissue, and undergo whatever chemical transformations are necessary to lead to mutation is largely related to the presence and activity of xenobiotic metabolizing enzymes (XMEs). A considerable number of polymorphisms in these enzymes have been described and their impact on human disease risk quantified. In general terms, compounds that downregulate enzymes involved in XME metabolism or upregulate clearance of dietary mutagens can prevent DNA damage. XME may be induced after exposure to xenobiotics, but the level to which this occurs, as well as their endogenous level of expression, is determined by genotype and varies in different organs and tissue. Although a considerable number of XMEs may be important for either enhancing or reducing the activity of dietary mutagens, two that have been extensively studied are cytochrome P450, which mediates oxidative processes, and glutathione S-transferases, which is involved in conjugation and nucleophilic trapping processes. Pool-Zobel et al. (65) and Hussain et al. (46) provide recent reviews of the impact of dietary factors on the expression and activity of these enzymes. Lampe (52) emphasizes the way in which individual variations in these enzymes affect the extent to which diet modulates the risk of chronic disease.

Nutrient Uptake, Metabolism, and Excretion

The protective effect of the homozygous variant TT form of the MTHFR genotype (C677T) on the risk of colorectal cancer seems to be modified by the level of methyl diets, i.e., by folate (49). In our pilot study on selenium in the Auckland population (48), the impact on DNA damage of selenium levels and of a dietary intervention with a preparation of selenized yeast appeared to vary according to certain elements of the genotype of the men in our volunteer group (L.R. Ferguson & M. Philpott, unpublished data). This reinforced the views of other workers, who have shown that genetic variability at codon 198 of human glutathione peroxidase 1 may determine response to selenium and significantly affect cancer risk. Yang et al. (80) considered the relative proportions of the wild-type Pro/Pro, heterozygous Pro/Leu, and homozygous Leu/Leu variants in a study of 315 lung cancer patients and 313 controls. They found a significantly higher proportion of the cases carried the variant allele. It should be noted, however, that this effect has not been repeated in other populations. In tissue culture studies, Hu & Diamond (45) confirmed the functionality of that variant by showing that cells carrying human glutathione peroxidase 1 proteins differing by a single amino acid at codon 198 respond differently to increasing selenium.

MTHFR: methylenetetrahydrofolate reductase

DECREASING DIET-RELATED MUTAGENESIS

As illustrated in the examples above, in vitro and animal tests confirm the presence of a wide range of mutagens and antimutagens in foods and food sources. The most important questions, however, are:

> Which are the most important mutagens in the human diet?
>
> How can individuals be protected against these mutagens?
>
> How do we estimate whether a given dietary regime will be detrimental or beneficial to human health?

Standard epidemiologic methods have given variable answers as to the human significance of dietary mutagens. Much of this work is summarized in considerable detail the AICR-WCRF

publication (1). Conventional epidemiology may show an association with a disease state such as cancer, with a type of food, and/or with a cooking process. However, this does not provide definitive proof of cause and effect. Because food is such a complex mixture, it is difficult, if not impossible, to attribute such results with certainty to any specific compound(s).

The field of molecular epidemiology was introduced in the early 1980s in order to overcome some of the limitations of more traditional methods. It relies on a molecular understanding of the events leading to disease providing a biomarker to enable early detection of changes in disease susceptibility (for a review, see 77). In this way, molecular epidemiology is particularly appropriate for assessing the impact of dietary changes before frank changes in disease status have time to occur. It is possible to utilize the distinctive mutations formed at unique nucleotide positions as molecular signatures that implicate specific classes of mutagen (or in a few cases, specific mutagens) in disease. By associating mutational spectra seen in the particular type of disease with the mutational spectra generated by known mutagens in experimental systems, it is at least theoretically possible to track backward to the source of the disease. Besaratinia & Pfeifer (12) outline the evidence for this approach to studying the etiology of cancer. The most successful example of this application of molecular epidemiology is from HCC, where the distinctive nature of mutations generated by the mycotoxin aflatoxin B1 has led to the conclusion that it is an important cause of the disease, although not the only one (46).

Not all chronic disease endpoints show clear molecular signatures, and alternative approaches are necessary to establish disease etiology. Mutagenicity testing and its variants, based on an understanding of the mechanisms leading to a mutagenic process, provide the basis of biomarker approaches to estimating human exposures in those tissues of interest. Even those components that do not form DNA adducts may nevertheless lead to DNA damage, and a measure of chromosome damage or DNA breakage per se may be a better indicator of exposure to such chemicals. The micronucleus assay is an indirect and relatively high throughput measure of chromosomal damage whose predictive potential for carcinogenesis has been recently validated in a large collaborative international study (15). The single-cell gel electrophoresis or COMET assay is also a highly sensitive measure of DNA damage that can be widely applied to different human tissues (20). These methods are especially appropriate where the dietary mutagen in question fails to provide a mutational signature. Ferguson (34) has reviewed the way in which biomarker studies can be integrated into an overall strategy for proving that a dietary intervention can modify disease risk, and the general approach is summarized in **Figure 4**.

A number of newer biomarkers rely on high-throughput technologies and "-omics" approaches, including transcriptomics, proteomics, and metabonomics, as well as the various methods being developed in epigenetics (25, 33). For example, Halliwell (42) summarized the problems with showing that flavonoids are likely to protect against cardiovascular disease, despite convincing evidence from a number of pre-experimental models. However, Guarrera et al. (41) were able to demonstrate that a flavonoid-rich diet was likely to have beneficial health effects through showing the diet's efficacy in modulating the expression of certain genes that have been clearly related to disease risk.

CONCLUSIONS

Diet itself is a complex mixture, and the impact of diet on mutagenesis is even more complex. Individual diets and dietary components have been studied for mutagenic effects in a wide range of systems, but technologies are only now becoming available to enable more comprehensive understanding of the interactions between different dietary components, let alone the complexity introduced by considering genotype. It will be important to consider the probability and implications of mutagenesis to

both population groups and to the individual. Although it is not possible to avoid mutagenic food components or dietary regimes, rational developments of antimutagens as chemopreventive agents coupled with technologies appropriate to nutrigenomics lead to an optimistic outlook for personalized nutrition protecting against diet-related mutagenesis in the future.

SUMMARY POINTS

1. Diet or dietary behavior plays an etiological role in chronic diseases.
2. The accumulation of different types of mutations is pivotal to the development and progression of chronic diseases, including cancer and cardiovascular disease.
3. In the carcinogenesis process, simple base-pair substitution mutations and/or epigenetic events are common at early stages, whereas structural chromosome changes, loss of heterozygosity, microsatellite instability, and gene or chromosome copy number variants signal a progressively dysfunctional cell.
4. Prominent among recognized dietary mutagens are a number of compounds that, usually following metabolic activation, generate a DNA-binding species leading to a distinct spectrum of base-pair substitution mutations. Generation of reactive species through endogenous or exogenous processes may also lead to DNA adduct formation along with strand breaks and other types of DNA damage.
5. Inhibitors of topoisomerase II enzymes, of DNA repair, or of the mitotic spindle may cause primarily structural chromosome changes, loss of heterozygosity, and copy number variants.
6. The action of dietary mutagens may be countered by antimutagens, many of which are derived from food plants.
7. The impact of both mutagens and antimutagens will be modulated by polymorphisms in genes associated with nutrient or xenobiotic uptake, distribution, and metabolism.
8. Biomarker approaches, utilizing mutagenicity test methods or the newer genomics technologies, will be essential to prove the efficacy of nutritional interventions that reduce mutagenesis in the human population.

FUTURE ISSUES

The following activities are recommended to reduce diet-related mutagenesis:

1. Comprehensively study mechanisms of dietary mutagenesis by means other than through DNA interactions to rationally stratify the relative risks associated with the diverse range of diet-related sources of mutation.
2. Develop more sensitive biomarkers to estimate an early stage of disease risk (and the efficacy of dietary interventions developed to protect against this risk) using the technologies appropriate to genomics/genetics and epigenetics.
3. Validate such molecular biomarkers for human use.
4. Optimize the study of gene-gene and gene-diet interactions.

5. Optimize the study of epigene-gene and epigene-diet interactions.
6. Develop strategies for optimizing nutrition at the individual level (personalized nutrition through nutrigenomics) to ensure optimal dietary delivery of nutrients and other potentially protective components.
7. Overcome the ethical implications of personalizing diets.
8. Educate the public as to where the real diet-associated risks lie, as distinct from imagined diet-associated risks, and how to avoid these risks.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the Auckland Cancer Society for financial support, Ms. Virginia Parslow for help with the figures, and Ms. Jacqueline Lyon for secretarial help.

LITERATURE CITED

1. Am. Inst. Cancer Res., World Cancer Res. Fund (AICR-WCRF). 2007. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*. Washington, DC: Am. Inst. Cancer Res.
2. Ames BN. 1979. Identifying environmental chemicals causing mutations and cancer. *Science* 204:587–93
3. Ames BN. 2006. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc. Natl. Acad. Sci. USA* 103:17589–94
4. Ames BN, Durston WE, Yamasaki E, Lee FD. 1973. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl. Acad. Sci. USA* 70:2281–85
5. Andreassi MG, Botto N, Colombo MG, Biagini A, Clerico A. 2000. Genetic instability and atherosclerosis: Can somatic mutations account for the development of cardiovascular diseases? *Environ. Mol. Mutagen.* 35:265–69
6. Bacic A, Harris PJ, Stone BA. 1988. Structure and function of plant cell walls. In *The Biochemistry of Plants*, ed. J Preiss, pp. 297–371. New York: Academic
7. Balkwill F, Mantovani A. 2001. Inflammation and cancer: back to Virchow? *Lancet* 357:539–45
8. Bandele OJ, Osheroff N. 2007. Bioflavonoids as poisons of human topoisomerase II alpha and II beta. *Biochemistry* 46:6097–108
9. Barbin A. 2000. Etheno-adduct-forming chemicals: from mutagenicity testing to tumor mutation spectra. *Mutat. Res.* 462:55–69
10. Bartsch H, Nair J. 2004. Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation-driven carcinogenesis. *Cancer Detect. Prev.* 28:385–91
11. Benditt EP, Benditt JM. 1973. Evidence for a monoclonal origin of human atherosclerotic plaques. *Proc. Natl. Acad. Sci. USA* 70:1753–56
12. Besaratinia A, Pfeifer GP. 2006. Investigating human cancer etiology by DNA lesion footprinting and mutagenicity analysis. *Carcinogenesis* 27:1526–37
13. Bingham SA, Pignatelli B, Pollock JR, Ellul A, Malaveille C, et al. 1996. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 17:515–23
14. Bodmer WF. 2006. Cancer genetics: colorectal cancer as a model. *J. Hum. Genet.* 51:391–96

15. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, et al. 2007. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* 28:625–31
16. Bridges BA, Bowyer DE, Hansen ES, Penn A, Wakabayashi K, eds. 1990. Report of ICPEMC Subcommittee 7/1. The possible involvement of somatic mutations in the development of atherosclerotic plaques (special issue). *Mutat. Res.* 239:143–87
17. Busby WFJ, Wogan GN. 1984. Aflatoxins. In *Chemical Carcinogens*, ed. C Searle, pp. 945–1136. Washington, DC: Am. Chem. Soc.
18. Calin GA, Croce CM. 2007. Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. *J. Clin. Invest.* 117:2059–66
19. Collins AR. 2001. Carotenoids and genomic stability. *Mutat. Res.* 475:21–28
20. Collins AR. 2005. Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols. *Am. J. Clin. Nutr.* 81:261–67S
21. Commoner B, Vithayathil AJ, Dolara P, Nair S, Madyastha P, Cuca GC. 1978. Formation of mutagens in beef and beef extract during cooking. *Science* 201:913–16
22. Courtemanche C, Huang AC, Elson-Schwab I, Kerry N, Ng BY, Ames BN. 2004. Folate deficiency and ionizing radiation cause DNA breaks in primary human lymphocytes: a comparison. *FASEB J.* 18:209–11
23. Coussens LM, Werb Z. 2002. Inflammation and cancer. *Nature* 420:860–67
24. Curfs DM, Knaapen AM, Pachen DM, Gijbels MJ, Lutgens E, et al. 2005. Polycyclic aromatic hydrocarbons induce an inflammatory atherosclerotic plaque phenotype irrespective of their DNA-binding properties. *FASEB J.* 19:1290–92
25. Davis CD, Ross SA. 2007. Dietary components impact histone modifications and cancer risk. *Nutr. Rev.* 65:88–94
26. Doll R, Peto R. 1981. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* 66:1191–308
27. Essigmann JM, Green CL, Croy RG, Fowler KW, Buchi GH, Wogan GN. 1983. Interactions of aflatoxin B1 and alkylating agents with DNA: structural and functional studies. *Cold Spring Harb. Symp. Quant. Biol.* 47(Pt. 1):327–37
28. Evans IA, Mason J. 1965. Carcinogenic activity of bracken. *Nature* 208:913–14
29. Exon JH. 2006. A review of the toxicology of acrylamide. *J. Toxicol. Environ. Health Pt. B Crit. Rev.* 9:397–412
30. Fearon ER, Vogelstein B. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759–67
31. Fenech M, Ferguson LR. 2001. Vitamins/minerals and genomic stability in humans. *Mutat. Res.* 475:1–6
32. Ferguson LR. 1998. Inhibitors of topoisomerase II enzymes: a unique group of environmental mutagens and carcinogens. *Mutat. Res.* 400:271–78
33. Ferguson LR. 2006. Nutrigenomics: integrating genomic approaches into nutrition research. *Mol. Diagn. Ther.* 10:101–8
34. Ferguson LR, ed. 2007. Biomarkers as endpoints in intervention studies. In *Molecular Epidemiology of Chronic Diseases: New Techniques and Approaches*, ed. P Vineis, S Garte, C Wild, pp. 253–64. Indianapolis, IN: Wiley
35. Ferguson LR, Barnett MPG, McNabb WC. 2007. Genetic, epigenetics, nutrition and chronic disease. *Agro-Food Ind. High Tech.* 3:49–51
36. Ferguson LR, Gregory TJ, Pearson AE, Hay JE, Lewis GD. 1996. Mutagenicity tests as a monitoring tool for potential mutagens and carcinogens in shellfish gathering areas of New Zealand. *N. Z. J. Marine Freshwater Res.* 30:413–21
37. Ferguson LR, Philpott M. 2007. Cancer prevention by dietary bioactive components that target the immune response. *Curr. Cancer Drug Targets* 7:459–64
38. Ferguson LR, Philpott M, Karunasinghe N. 2004. Dietary cancer and prevention using antimutagens. *Toxicology* 198:147–59
39. Ferruzzi MG, Blakeslee J. 2007. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutr. Res.* 27:1–12
40. Fu PP, Xia Q, Lin G, Chou MW. 2004. Pyrrolizidine alkaloids—genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. *Drug Metab. Rev.* 36:1–55

41. Guarrera S, Sacerdote C, Fiorini L, Marsala R, Polidoro S, et al. 2007. Expression of DNA repair and metabolic genes in response to a flavonoid-rich diet. *Br. J. Nutr.* 98:525–33
42. Halliwell B. 2007. Dietary polyphenols: good, bad, or indifferent for your health? *Cardiovasc. Res.* 73:341–47
43. Halliwell B. 2007. Oxidative stress and cancer: Have we moved forward? *Biochem. J.* 401:1–11
44. Harris PJ, Ferguson LR. 1999. Dietary fibres may protect or enhance carcinogenesis. *Mutat. Res.* 443:95–110
45. Hu YJ, Diamond AM. 2003. Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. *Cancer Res.* 63:3347–51
46. Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. 2007. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene* 26:2166–76
47. Izzotti A, De Flora S, Petrilli GL, Gallagher J, Rojas M, et al. 1995. Cancer biomarkers in human atherosclerotic lesions: detection of DNA adducts. *Cancer Epidemiol. Biomarkers Prev.* 4:105–10
48. Karunasinghe N, Ryan J, Tuckey J, Masters J, Jamieson M, et al. 2004. DNA stability and serum selenium levels in a high-risk group for prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* 13:391–97
49. Kim DH. 2007. The interactive effect of methyl-group diet and polymorphism of methylenetetrahydrofolate reductase on the risk of colorectal cancer. *Mutat. Res.* 622:14–18
50. Kim HS, Kwack SJ, Lee BM. 2000. Lipid peroxidation, antioxidant enzymes, and benzo[a]pyrene-quinones in the blood of rats treated with benzo[a]pyrene. *Chem. Biol. Interact.* 127:139–50
51. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, et al. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* 113(Suppl. 9B):71–88S
52. Lampe JW. 2007. Diet, genetic polymorphisms, detoxification, and health risks. *Altern. Ther. Health Med.* 13:S108–11
53. Leblanc JC, Tard A, Volatier JL, Verger P. 2005. Estimated dietary exposure to principal food mycotoxins from the first French Total Diet Study. *Food Addit. Contam.* 22:652–72
54. Lijinsky W. 1999. N-nitroso compounds in the diet. *Mutat. Res.* 443:129–38
55. Mei N, Guo L, Fu PP, Heflich RH, Chen T. 2005. Mutagenicity of comfrey (*Symphytum Officinale*) in rat liver. *Br. J. Cancer* 92:873–75
56. Mirvish SS, Wallcave L, Eagen M, Shubik P. 1972. Ascorbate-nitrite reaction: possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science* 177:65–68
57. Nagao M, Honda M, Seino Y, Yahagi T, Sugimura T. 1977. Mutagenicities of smoke condensates and the charred surface of fish and meat. *Cancer Lett.* 2:221–26
58. Nair J, De Flora S, Izzotti A, Bartsch H. 2007. Lipid peroxidation-derived etheno-DNA adducts in human atherosclerotic lesions. *Mutat. Res.* 621:95–105
59. Nakai Y, Nelson WG, De Marzo AM. 2007. The dietary charred meat carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine acts as both a tumor initiator and promoter in the rat ventral prostate. *Cancer Res.* 67:1378–84
60. Ostertag W, Duisberg E, Sturmann M. 1965. The mutagenic activity of caffeine in man. *Mutat. Res.* 2:293–96
61. Pavanello S, Pulliero A, Saia BO, Clonfero E. 2006. Determinants of *anti*-benzo[a]pyrene diol epoxide–DNA adduct formation in lymphomonocytes of the general population. *Mutat. Res.* 611:54–63
62. Penn A, Snyder C. 1988. Arteriosclerotic plaque development is "promoted" by polynuclear aromatic hydrocarbons. *Carcinogenesis* 9:2185–89
63. Pfohl-Leszkowicz A, Manderville RA. 2007. Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. *Mol. Nutr. Food Res.* 51:61–99
64. Phillips DH. 1999. Polycyclic aromatic hydrocarbons in the diet. *Mutat. Res.* 443:139–47
65. Pool-Zobel B, Veeriah S, Bohmer FD. 2005. Modulation of xenobiotic metabolising enzymes by anticarcinogens—focus on glutathione S-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis. *Mutat. Res.* 591:74–92
66. Porta M, Vioque J, Ayude D, Alguacil J, Jariod M, et al. 2003. Coffee drinking: the rationale for treating it as a potential effect modifier of carcinogenic exposures. *Eur. J. Epidemiol.* 18:289–98

67. Prakash AS, Pereira TN, Reilly PE, Seawright AA. 1999. Pyrrolizidine alkaloids in human diet. *Mutat. Res.* 443:53–67
68. Ross JA, Potter JD, Robison LL. 1994. Infant leukemia, topoisomerase II inhibitors, and the MLL gene. *J. Natl. Cancer Inst.* 86:1678–80
69. Ross JS, Stagliano NE, Donovan MJ, Breitbart RE, Ginsburg GS. 2001. Atherosclerosis and cancer: common molecular pathways of disease development and progression. *Ann. N. Y. Acad. Sci.* 947:271–92; discussion 92–93
70. Shahin M, Smith BL, Prakash AS. 1999. Bracken carcinogens in the human diet. *Mutat. Res.* 443:69–79
71. Shelling AN, Ferguson LR. 2007. Genetic variation in human disease and a new role for copy number variants. *Mutat. Res.* 622:33–41
72. Smela ME, Currier SS, Bailey EA, Essigmann JM. 2001. The chemistry and biology of aflatoxin B(1): from mutational spectrometry to carcinogenesis. *Carcinogenesis* 22:535–45
73. Steiner C, Peters WH, Gallagher EP, Magee P, Rowland I, Pool-Zobel BL. 2007. Genistein protects human mammary epithelial cells from benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide and 4-hydroxy-2-nonenal genotoxicity by modulating the glutathione/glutathione S-transferase system. *Carcinogenesis* 28:738–48
74. Sugimura T. 1982. Mutagens, carcinogens, and tumor promoters in our daily food. *Cancer* 49:1970–84
75. Turesky RJ. 2007. Formation and biochemistry of carcinogenic heterocyclic aromatic amines in cooked meats. *Toxicol. Lett.* 168:219–27
76. van Waalwijk van Doorn-Khosrovani SB, Janssen J, Maas LM, Godschalk RW, Nijhuis JG, van Schooten FJ. 2007. Dietary flavonoids induce MLL translocations in primary human CD34+ cells. *Carcinogenesis* 28:1703–9
77. Vineis P, Perera F. 2007. Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. *Cancer Epidemiol. Biomarkers Prev.* 16:1954–65
78. Waters DJ, Shen S, Glickman LT, Cooley DM, Bostwick DG, et al. 2005. Prostate cancer risk and DNA damage: translational significance of selenium supplementation in a canine model. *Carcinogenesis* 26:1256–62
79. Wiseman H, Halliwell B. 1996. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem. J.* 313:17–29
80. Yang P, Bamlet WR, Ebbert JO, Taylor WR, de Andrade M. 2004. Glutathione pathway genes and lung cancer risk in young and old populations. *Carcinogenesis* 25:1935–44
81. Yun CH, Jung U, Son CG, Ju HR, Han SH. 2006. 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), a food-borne carcinogenic heterocyclic amine, promotes nitric oxide production in murine macrophages. *Toxicol. Lett.* 161:18–26

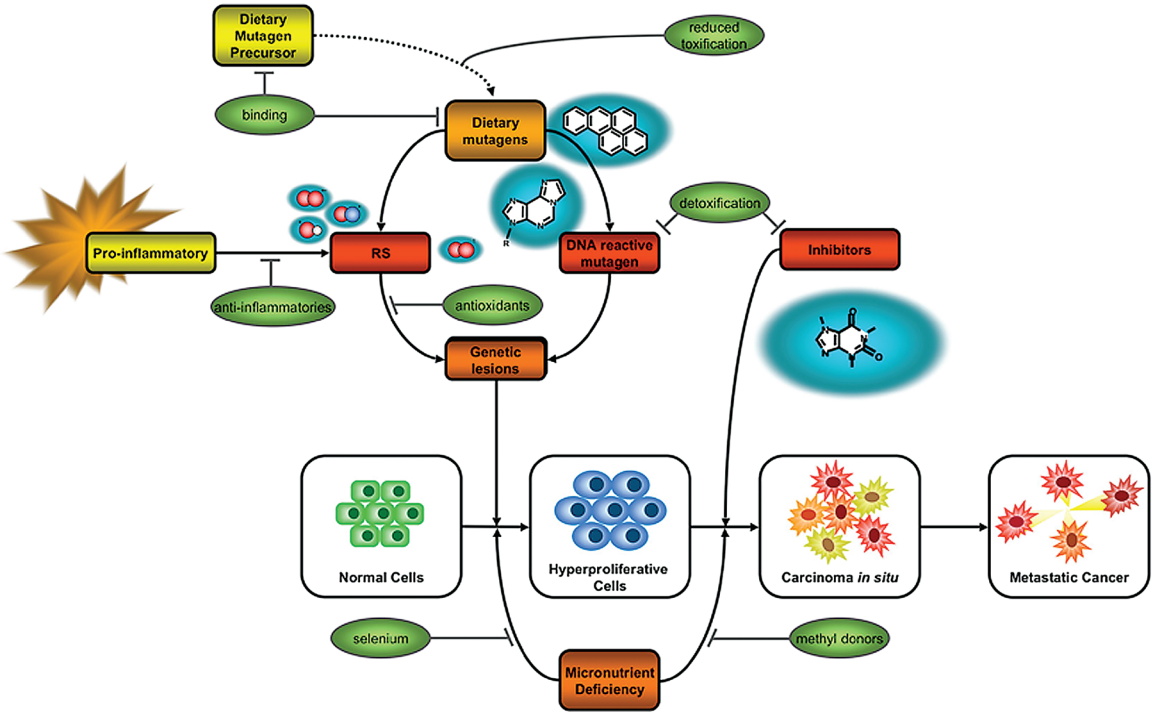

Figure 1

Role of dietary mutagens in carcinogenesis. Dietary mutagens, which may arise from the metabolism of nonmutagenic precursors, fall into several broad categories: mutagens that react with DNA directly; reactive species (RS); compounds that give rise to RS as a consequence of triggering inflammation; and inhibitors of processes such as mitotic spindle formation, topoisomerase II enzymes, or DNA repair enzymes. The ensuing DNA damage can occur at any point during carcinogenesis, although DNA-reactive mutagens and those acting as RS often initiate events, whereas those acting as inhibitors tend to drive progression. Specific micronutrient deficiencies can impair defense mechanisms that would otherwise prevent or repair DNA damage, or alter promoter methylation of oncogenes and tumor-suppressor genes, thus promoting carcinogenesis. Green ovals depict dietary antimutagens, which may act in a number of ways: Dietary fiber can bind mutagens or their precursors in the gut and prevent their absorption. Dietary antimutagens can inhibit the enzymatic conversion of mutagen precursors into mutagens. Anti-inflammatory compounds can prevent the production of RS that are a result of inflammation, and antioxidants can neutralize RS. Compounds that upregulate enzymes involved in the metabolism and clearance of dietary mutagens can prevent DNA damage. Selenium is incorporated into several antioxidant enzymes that are a key part of RS defenses. Dietary methyl donors are essential for the maintenance of promoter methylation.

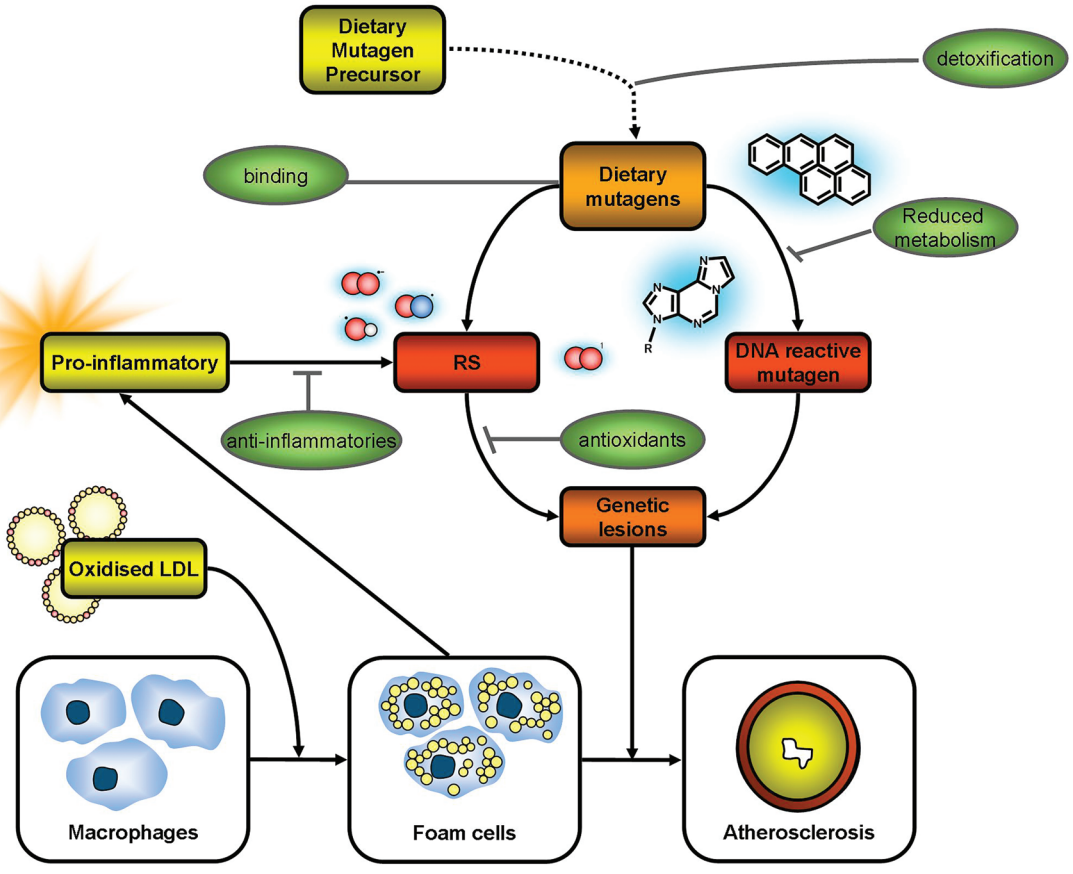

Figure 2

Role of dietary mutagens in atherosclerosis. Dietary mutagens, which may arise from the metabolism of nonmutagenic precursors, fall into several broad categories: mutagens that react with DNA directly; reactive species (RS); and compounds that give rise to RS as a consequence of triggering inflammation. The ensuing DNA damage results in monoclonal expansion of mutated cells, giving rise to atherosclerotic plaque. Green ovals depict dietary antimutagens, which may act in a number of ways: Dietary fiber can bind mutagens or their precursors in the gut and prevent their absorption. Dietary antimutagens can inhibit the enzymatic conversion of mutagen precursors into mutagens. Anti-inflammatory compounds can prevent the production of RS that are a result of inflammation, and antioxidants can neutralize RS. Compounds that upregulate enzymes involved in the metabolism and clearance of dietary mutagens can prevent DNA damage.

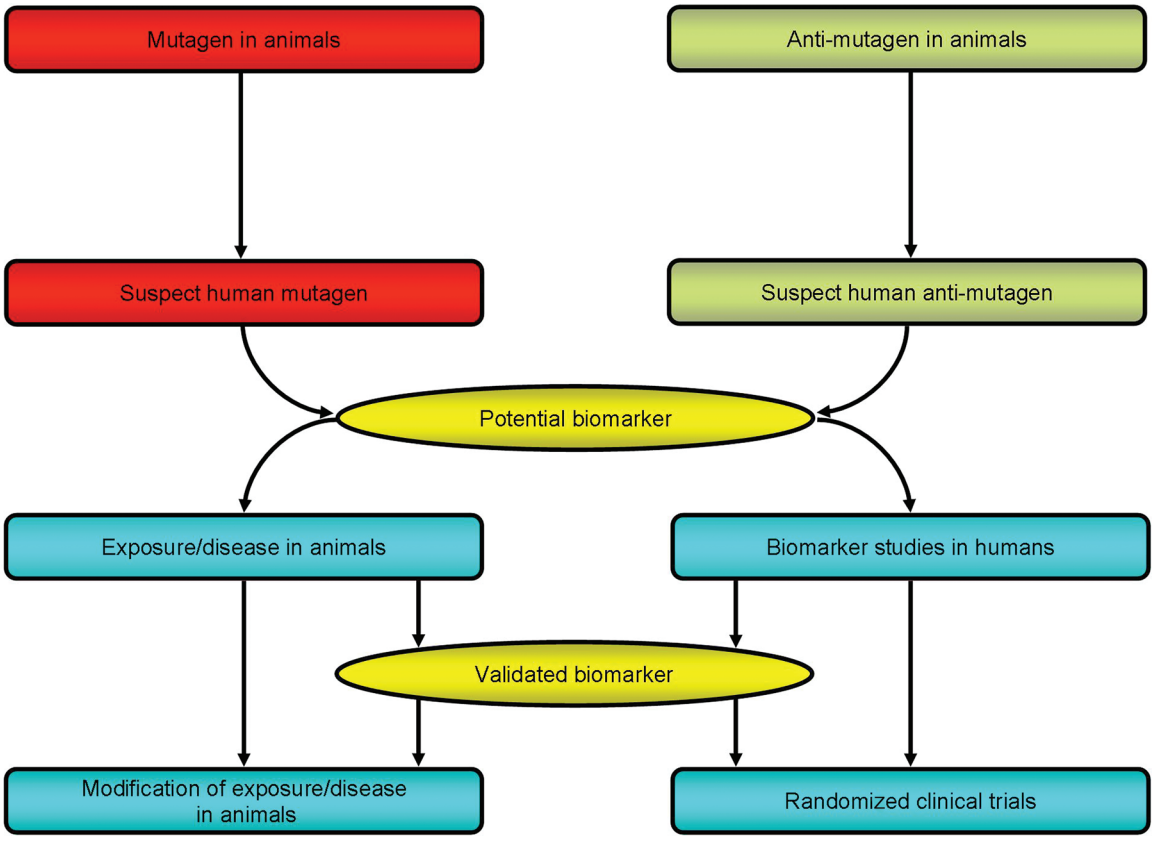

Figure 4

Methods for studying dietary mutagens as putative human dietary carcinogens.

Annual Review of Nutrition

Volume 28, 2008

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Nutrition* articles may be found at http://nutr.annualreviews.org/errata.shtml