Annu. Rev. Nutr. 2000. 20:485–505

DIET AND APOPTOSIS

Walter H. Watson, Jiyang Cai, and Dean P. Jones
Departments of Biochemistry and Ophthalmology, Emory University School of Medicine, Atlanta, Georgia 30322

Key Words Phase 2 enzyme inducers, isoflavones, zinc, atherosclerosis, immunity

■ **Abstract** A range of compounds in or derived from the diet modulates apoptosis in cell cultures in vitro. These observations have important implications concerning the mechanisms whereby dietary components affect health. Proapoptotic compounds could protect against cancer by enhancing elimination of initiated, precancerous cells, and antiapoptotic compounds could promote tumor formation by inhibiting apoptosis in genetically damaged cells. Proapoptotic compounds could also contribute to age-related degenerative diseases by activating cell death in postmitotic cells or shifting the normal balance of mitosis and apoptosis in tissues with regenerative capacity. Many age-related diseases, for example macular degeneration and Parkinson's disease, appear to have oxidative stress as an underlying component that interacts with genetic, dietary, and environmental factors to determine relative risk in an individual. Oxidative stress activates apoptosis, and antioxidants protect against apoptosis in vitro; thus, a central role of dietary antioxidants may be to protect against apoptosis. However, little in vivo data are available to directly link diet with altered apoptosis as an underlying determinant of disease. Moreover, the possible antagonistic effects of different dietary components and the uncertainty about whether proapoptotic compounds that may protect against cancer could contribute to degenerative diseases and vice versa indicate that there is a great need for better in vivo assessment of apoptosis and that caution should be exercised when extrapolating in vitro data on apoptosis to in vivo dietary recommendations.

CONTENTS

INTRODUCTION 486
DEFINITION AND FUNCTIONS OF APOPTOSIS 486
CARCINOGENESIS 488
Phase 2 Enzyme Inducers as Activators of Apoptosis 489
Butyric Acid 490
Isoflavones 491
Monoterpenes 491
Dietary Fat 492
Sphingolipids 492
NEURODEGENERATIVE DISEASES 493
Antioxidants 494

AGE-RELATED MACULAR DEGENERATION 494
Antioxidants 495
ATHEROSCLEROSIS 495
Antioxidants 496
Cholesterol 497
Zinc 497
IMMUNITY 497
Zinc 498
FUTURE DIRECTIONS 498

INTRODUCTION

Apoptosis plays a fundamental role in maintenance of tissues and organ systems by providing a controlled cell deletion to balance cell proliferation. It is now clear that altered rates of apoptosis are central to many disease processes. Accumulating evidence shows that dietary factors can either activate or inhibit apoptosis. Thus, dietary effects on apoptosis are likely to be among the central mechanisms underlying associations between diet and disease. In this brief review, we summarize recent studies on dietary components and apoptosis in the context of some specific disease processes.

DEFINITION AND FUNCTIONS OF APOPTOSIS

Controlled cell elimination or programmed cell death during development has been known for at least a century. This process was later recognized to be morphologically similar to cell death seen under some pathologic and toxicologic conditions, and it has been termed "apoptosis" to distinguish it from the common form of tissue injury, known as "necrosis" (54).

Homeostasis in multicellular organisms is a balance between cell division and elimination. Both processes are tightly regulated to allow development, acclimatization to new environmental conditions, and protection against toxicologic and pathologic stresses. Factors that enhance the rate of apoptosis can contribute to degenerative diseases such as Parkinson's disease, whereas factors that inhibit apoptosis can lead to proliferative diseases such as cancer (126).

During the past several years, the central biochemical pathway that mediates apoptosis has been elucidated (102). This pathway involves proteolytic enzymes known as caspases that cleave adjacent to aspartic acid residues in conserved amino acid sequences of target proteins. The general mechanism is conserved in animal species, and, in higher organisms, involves "initiator caspases" and "effector caspases" that work together to form a proteolytic cascade (21; Figure 1). Caspases exist in cells in precursor forms (procaspases) that have a low proteolytic activity. In response to various stimuli, initiator procaspases undergo selective proteolysis with an associated increase in catalytic activity. Initiator caspases cleave

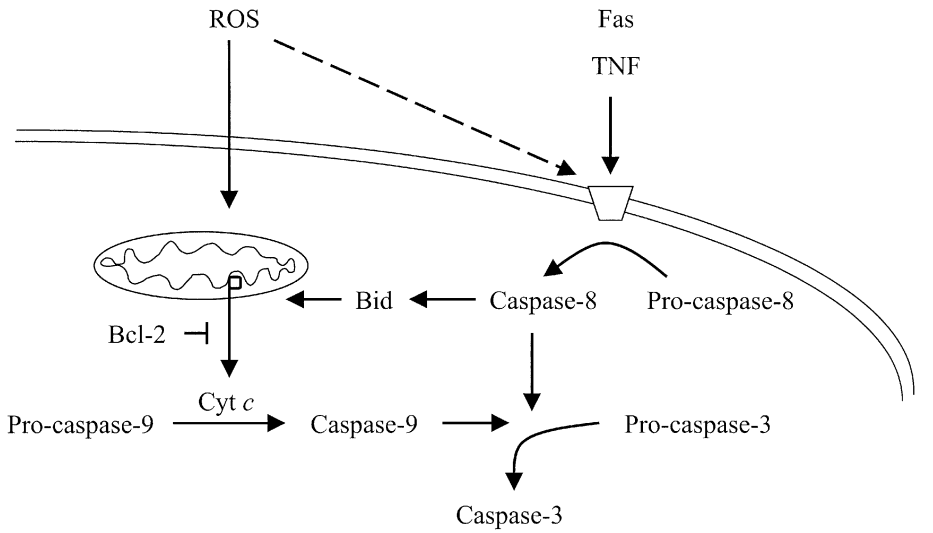

Figure 1 Apoptotic pathways. ROS, Reactive oxygen species; Cyt *c*, cytochrome *c*; TNF, tumor necrosis factor.

and activate effector caspases, which then cleave target proteins and result in the characteristic biochemical and morphologic changes of apoptosis. For instance, cleavage of a DNase inhibitor allows DNA cleavage into oligonucleosome-length fragments, cleavage of poly-(ADP-ribose) polymerase results in inhibition of DNA repair, cleavage of nuclear lamins contributes to condensation of nuclei, cleavage of cytoskeletal components allows bleb formation, and cleavage of fodrin contributes to the appearance of the phagocytosis-signaling phosphatidylserine on the cell surface (102). Cell-specific expression of different target proteins results in some variation in characteristics so that not all cells with activation of caspases share all of the morphologic features.

Regulation of apoptosis involves both proapoptotic and antiapoptotic components. Genetic studies in *Caenorhabditis elegans* identified ced-3 as essential for apoptosis, ced-4 as involved in activation, and ced-9 as involved in inhibition of apoptosis. At least 13 mammalian homologs of ced-3 (21), 2 homologs of ced-4 (67), and 12 homologs of ced-9 (1) have been identified. The basic features are conserved in higher organisms, but more specialized systems with greater complexity have evolved. Consequently, one can expect dietary effects on apoptosis in human tissues and cell types to be selective owing to this complexity.

One of the best-characterized activation mechanisms is associated with mitochondria (17). Apoptosis is activated when mitochondria release cytochrome *c*, which binds to the Ced-4 homolog Apaf-1 (67). This binding, in the presence of dATP, facilitates cleavage of the initiator procaspase-9. Caspase-9 cleaves effector caspases to execute apoptosis. The process is blocked by homologs of Ced-9,

such as Bcl-2 and Bcl-xL, which are antiapoptotic members of the Bcl-2 family (102). Regulation of this antiapoptotic function involves an antagonistic action of proapoptotic members of the Bcl-2 family, such as Bax (1) or associated proteins such as Bid (73). These proapoptotic proteins can function to amplify an apoptotic signal and also can activate apoptosis directly by inducing mitochondrial swelling (67) and release of cytochrome *c* (73). Dietary agents such as ceramide can activate apoptosis by inducing cytochrome *c* release from the mitochondria (113).

A second well-characterized activation mechanism involves death receptors in the plasma membrane. These receptors are members of the tumor necrosis factor (TNF) superfamily that contain a "death domain." At least five receptors of this type are known, with the Fas receptor being among the best characterized (4). Fas-mediated apoptosis occurs when binding of an appropriate extracellular ligand promotes trimerization of the receptor. This oligomerization facilitates binding of intracellular signaling molecules into a complex that includes adaptor protein FADD (Fas-associated death domain) and a protein termed FLASH that has a sequence similar to that of Ced-4 (49). Recruitment and cleavage of the initiator procaspase 8 by this complex activates the caspase cascade.

Regulation of this activation mechanism is complex. Both Fas and the physiological Fas ligand (FasL) are under transcriptional regulation with increased expression after exposure to oxidants (50, 120). Thiol antioxidants, such as *N*-acetylcysteine and glutathione, block this increase and inhibit apoptosis. Because antioxidants interact with each other in these effects [e.g. glutathione level is increased in association with increased ascorbate or α-tocopherol (51, 52)], antioxidants are likely to suppress Fas-mediated apoptosis. A variety of other indirect effects of dietary factors are possible owing to control mechanisms that involve viral proteins and systems that integrate cell cycle control and other aspects of intracellular signaling (31).

CARCINOGENESIS

Epidemiologic and animal studies have identified associations between certain diets and cancer risk (11). Carcinogenesis is a multistage process in which a normal cell is transformed into a lineage with a malignant phenotype (see Figure 2). Cells are initiated by the acquisition of an activating mutation in an oncogene or an inactivating mutation in a tumor suppressor gene. A promotion phase confers a growth advantage to initiated cells, and cells accumulate abnormal characteristics, ultimately progressing to a metastatic tumor. Diet can affect the overall process of carcinogenesis by different mechanisms. Because apoptosis provides a physiologic mechanism for elimination of abnormal cells, dietary factors affecting apoptosis could have important effects on carcinogenesis.

Activation of apoptosis in precancerous cells offers a mechanism for dietary prevention of cancer. An initiated cell does not always go on to become a malignant cancer cell; most initiated cells are deleted and never form a tumor (126).

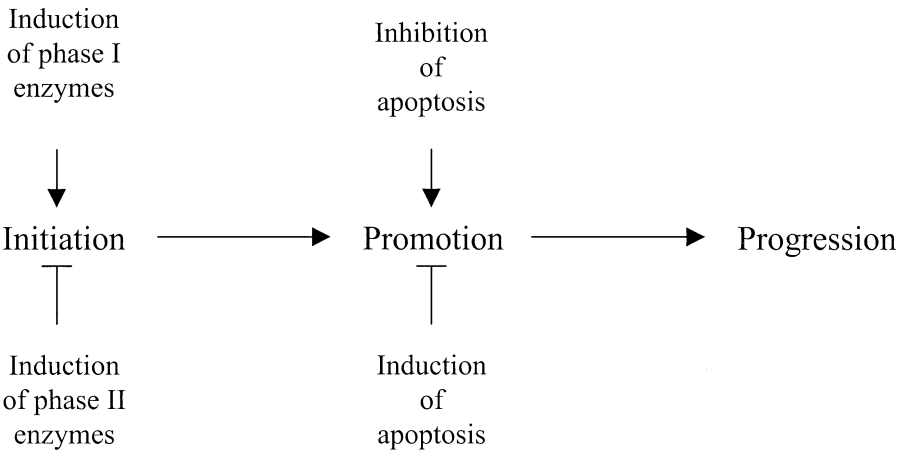

Figure 2 Multistage model of carcinogenesis.

Genetic mutations that affect cell growth and proliferation are normally recognized by the cell at one of several cell cycle checkpoints. The tumor suppressor gene *p53* activates the apoptotic pathway in response to DNA damage or dysregulated expression of cell cycle genes (8, 61). Anticarcinogenic dietary components may function to increase expression of proapoptotic components in initiated proliferating cells and thereby prevent or delay development of a tumor.

Inhibition of apoptosis can allow tumor development. In animal models, most chemical initiators do not cause tumors unless a promoter is given subsequent to the application of the initiator. Many tumor promoters inhibit apoptosis in vitro (131). Thus, a generalized way in which diet can affect cancer is to inhibit apoptosis in preneoplastic cells. This would allow these cells to survive, proliferate, and further acquire more aggressive growth characteristics. The potential antagonistic effects of different dietary factors poses a major challenge for extrapolating results from single dietary components studied in vitro to recommendations for consumption of foods to reduce cancer risk.

Phase 2 Enzyme Inducers as Activators of Apoptosis

Many carcinogens (or initiators) must be metabolically transformed into the ultimate initiating species by Phase 1 enzymes such as cytochrome P450 (Figure 3). Agents that increase the activity of cytochrome P450 isozymes can increase the initiation potential of a carcinogen. In contrast, Phase 2 enzymes such as glutathione-*S*-transferase and UDP-glucuronosyltransferase detoxify and/or enhance elimination of toxic species. Agents that induce Phase 2 enzymes tend to suppress initiation. Several Phase 2 detoxification enzyme inducers found in common foods have been shown to be protective against carcinogenesis in animal models (42, 97, 111, 115). However, recent evidence suggests that increased clearance of

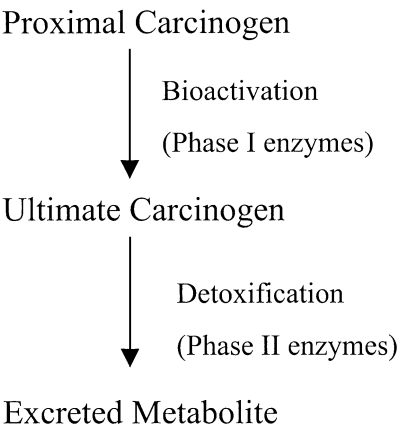

Figure 3 Bioactivation of carcinogens.

genotoxic metabolites is not the only mechanism by which these compounds can prevent cancer. In the human colon cancer cell line HT29, benzylisothiocyanate, allyl sulfide, dimethylfumarate, and butylated hydroxyanisole induced apoptosis in the same concentration range that induced glutathione-*S*-transferase (55). Isothiocyanates induced apoptosis via a caspase-3–dependent mechanism in HeLa cells, Jurkat T cells, and human embryonic kidney 293 cells (20, 134). Sakamoto et al found that allyl sulfides induced apoptosis in neoplastic human lung cells but not in non-neoplastic lung cells (101). Thus, in addition to protection against initiation, these chemopreventive agents may also block the promotion/progression stages of carcinogenesis by inducing apoptosis. Compounds with both activities may be particularly potent as cancer preventive agents.

Butyric Acid

High-fiber diets are associated with decreased risk for colon cancer (11, 76). Although there are several mechanisms whereby fiber could protect against carcinogenesis, considerable interest has focused on a metabolic product of fiber, butyrate. Fiber is metabolized by bacteria within the lumen of the gut to butyrate and other short-chain fatty acids, such as propionate and acetate. Butyrate is a primary energy source for colonic epithelial cells (71), but its role in chemoprevention may involve mechanisms that are unrelated to this function. Butyrate is an inhibitor of histone deacetylase. Histone hyperacetylation and the resultant increased transcription may account for butyrate induction of Phase 2 detoxification enzymes (56). Alternatively, the transcriptional activation may be a secondary consequence of oxidation of cellular thiol pools (56). At similar concentration ranges, butyrate and its analog triglyceride have been found to induce apoptosis in cultured colorectal (32, 44, 55), lymphoid (80), and mammary carcinoma cells (43). Butyrate also potentiated the apoptotic effects of the Phase 2 enzyme inducers discussed above (55).

Butyrate induces terminal differentiation in colorectal, prostate, and lymphoid cells. Terminally differentiated cells accumulate mitochondria with altered ultrastructures (74) that can, in principle, lead to mitochondrial depolarization, release of cytochrome *c*, and activation of apoptosis. A recent report showed that expression of Fas and FasL was increased after butyrate treatment and that chimeric soluble Fas receptor protein inhibited butyrate-induced apoptosis (32). Thus, the results indicate that butyrate, and therefore fiber, could induce apoptosis by either a mitochondrial or a death receptor-dependent mechanism. Additional experiments are needed to determine whether provision of butyrate or butyrate-producing foods enhances apoptosis in vivo and whether this preferentially targets precancerous cells.

Isoflavones

Consumption of diets that are high in soybeans is associated with decreased cancer risk in human populations (105). High soy diets also inhibit tumor development in animal models. Efforts to identify the active components of soy have identified isoflavones such as genistein as likely candidates. Genistein inhibits tumor formation in animal models of breast (6, 34), prostate (90), and bladder cancer (137). In vitro, genistein induces apoptosis of lung cancer cells (70), breast cancer cells (69), bladder cancer cells (137), and human prostate cancer cells (89, 136). Several mechanisms have been proposed to explain the anticarcinogenic and proapoptotic effects of genistein. Genistein is an inhibitor of protein tyrosine kinases (2), topoisomerase II (79), and angiogenesis (136). Proapoptotic factors such as Bax and p21WAF1/CIP1 are induced by genistein in breast cancer cells (69, 108) and in prostate cancer cells (24). Furthermore, the antiapoptotic factor Bcl-2 is inhibited by genistein in breast cancer cells (69, 22). Thus, isoflavones can inhibit the promotion/progression stage of cancer, at least in part, by deleting damaged or initiated cells. Although more specific in vivo experiments are needed to test this hypothesis, such an effect may provide a mechanistic explanation for the observation that regular consumption of diets that are high in soy products is associated with decreased risk of many different cancers.

Monoterpenes

Monoterpenes such as limonene and perillyl alcohol are abundant components of citrus oils, cherries, and mint (7, 23, 37). These compounds inhibit both initiation and promotion in animal models of tumorigenesis and can induce regression of established rodent mammary and pancreatic tumors. Mills et al (83) reported that perillyl alcohol prevented hepatocarcinogenesis at the level of promotion and that this inhibition was associated with an increased rate of apoptosis in the liver. Proposed mechanisms for the chemotherapeutic and chemopreventive properties include inhibition of protein prenylation and induction of *c-Jun*, transforming growth factor β (TGF-β), TGF-β receptor, and mannose-6-phosphate/insulin-like grrowth factor II receptor (M6P/IGF2R). Each of these effects can potentially influence apoptotic pathways. *c-Jun*, as part of the heterodimeric transcription factor

AP-1, plays an important role in cell growth and cell death (65). Isoprenylation of Ras and other proteins is required for normal signal transduction (35). TGF-β is an important growth factor that can induce apoptosis in transformed cells (41). Loss-of-function mutations in the *M6P/IGF2R* gene are associated with many tumors, indicating that this gene is probably a tumor suppressor (88). Limonene induced glutathione-*S*-transferase and UDP-glucuronyl transferase at concentrations that inhibited chemically induced tumors in rats (30), indicating that limonene may inhibit initiation by induction of Phase 2 detoxification enzymes, as discussed above. However, the effects on promotion and tumor regression appear more likely to be a consequence of enhanced apoptosis. Thus, inhibition of tumor formation in animals by monoterpenes occurs through different mechanisms, which may include induction of apoptosis in precancerous cells.

Dietary Fat

The relationship between dietary-fat intake and cancer incidence has been the subject of several epidemiological studies (60, 66, 71). Although there is some disagreement among these studies, the strongest associations are between dietary fat and colon, breast, and prostate cancer. Both the amount and type of fat consumed are important determinants of cancer risk. For example, diets high in saturated fats may increase the risk of cancer, whereas diets high in polyunsaturated fats that are rich in omega-3 fatty acids (e.g. from fish and fish oil) may decrease the risk of cancer (71, 127). Results from animal studies largely support the epidemiological evidence; both the amount and type of dietary fat affect carcinogenesis. Reddy and coworkers have described a role for apoptosis in colon cancer based on changes in phospholipid turnover and arachadonic acid metabolism (reviewed in 71). Saturated fats induce the release of arachadonic acid, a substrate for cyclooxygenase (COX). Inducible COX-2 activity is associated with an inhibition of apoptosis. Indeed, COX-2 expression is elevated in human colorectal carcinomas and in the colonic mucosa and tumors of rats (71). Conversely, fish oil, which is rich in omega-3 fatty acids, inhibits tumor development in animal models and increases the rate of apoptosis (12, 19, 62). Thus, although the data are insufficient to draw a strong causal link, accumulating evidence indicates that effects of dietary fats on apoptosis may contribute to risk of colorectal cancer and indicate that more detailed mechanistic studies are needed.

Sphingolipids

Sphingolipids are prevalent in the diet and are important constituents of membranes and other lipid-rich structures in the body (81, 129). There have been no epidemiological or clinical studies addressing the relationship between dietary sphingolipids and cancer. However, sphingolipids are high in dairy and soy products, both of which have anticarcinogenic properties (91, 105). In animal models, sphingolipids protect against colon cancer. In the colon, dietary sphingomyelin is converted to ceramide and sphingosine by the successive actions of sphingomyelinase and

ceramidase. Ceramide and sphingosine function as intracellular second messengers and, in general, promote growth arrest and apoptosis. In human and rat colon tumors, sphingomyelinase expression is lower than in normal colonic epithelial cells. Therefore, dietary sphingomyelin would have to act on cancer cells at an early stage of development, before sphingomyelinase expression is down-regulated (71). Alternatively, ceramide or water-soluble ceramide analogs, which induce apoptosis in transformed cell lines (129), may induce apoptosis at later stages of tumor development. Clearly, clinical and epidemiologic studies are needed to address the possible role of sphingolipid-induced apoptosis as a cancer preventive mechanism.

NEURODEGENERATIVE DISEASES

An imbalance between apoptosis and mitosis probably contributes to the age-related decline in function of most organ systems. The relative contributions of declining mitosis and increased apoptosis remain unclear for many tissues. However, some tissues, including regions of the brain, are largely postmitotic. Such tissues could be adversely affected by even a small change in the rate of apoptosis, especially over the course of a lifetime (25).

The role of apoptosis in the death of neurons in neurodegenerative diseases has been the subject of several recent reviews (3, 14, 27, 33, 82, 117, 125). Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis are characterized by the loss of specific populations of neurons (82). Although the evidence is not yet conclusive, postmortem examinations, animal models, and cell culture experiments indicate that apoptosis is the predominant mechanism of death in degenerating neurons.

Both genetic and environmental factors can affect the rate of neuronal apoptosis. For example, early onset Parkinson's disease has a strong genetic component, whereas late-onset Parkinson's disease (age of onset >50 years) is probably more dependent on environmental factors (122). Similarly, familial and sporadic forms of Alzheimer's disease are differentially dependent on hereditary factors (13). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a contaminant in the production of heroin, produces strikingly Parkinson's-like symptoms in humans and laboratory animals. MPTP has proven to be a useful laboratory tool in the study of Parkinson's disease and has highlighted the possibility that environmental toxins could induce or accelerate the disease (95).

A great deal of evidence suggests that oxidative stress contributes to the development of neurodegenerative diseases. Postmortem evaluation of brains from patients with Parkinson's disease reveals evidence of lipid peroxidation, protein oxidation, and DNA oxidation. Also, increased iron, in the absence of an increase in ferritin, and decreased glutathione concentrations suggest that the substantia nigra is in a state of oxidative stress (5, 75). Mutations in the antioxidant enzyme copper/zinc superoxide dismutase (SOD) are found in $\sim$20% of cases of familial

amyotropic lateral sclerosis, and expression of these mutant SODs in PC12 cells induced cell death that was typical of apoptosis (36). The amyloid beta peptide, implicated in Alzheimer's disease, induced oxidative stress and apoptosis in cultured neurons (93, 133).

Mitochondria may represent the source of both oxidative species and mediators of apoptosis in degenerating neurons. Parkinson's disease is associated with a defect in complex I of the electron transport chain in the mitochondria, which can result in the increased release of reactive oxygen species from the mitochondria and can modulate the opening of the permeability transition pore (124). 1-Methyl-4-phenylpyridinium (MPP+), the active metabolite of MPTP, is a complex I inhibitor that induces caspase-dependent apoptosis in cultured neurons by inducing the mitochondrial permeability transition and the subsequent release of cytochrome *c* into the cytosol (28, 29). Studies in transgenic mice have shown that MPTP-induced neuronal apoptosis is inhibited in mice that express a dominant negative mutant of interleukin-1β-converting enzyme (57) and in mice overexpressing the antiapoptotic protein Bcl-2 (132).

Antioxidants

Because oxidative stress can activate apoptosis, it is conceivable that increased consumption of antioxidants could slow the rate of neuronal death and delay the onset of symptoms. Vitamin E inhibited apoptosis of striatal neurons grown in culture (48) and prevented neuronal degeneration induced by amyloid beta peptide in vitro (92, 133). A clinical trial in patients with Alzheimer's disease showed that vitamin E (2000 IU/day) delayed the progression of the disease by 6 months, but had no effect on cognitive function (77). However, the same dose of vitamin E had no effect on the progression of Parkinson's disease (110). It has been proposed that a combination of different antioxidants would be more effective (94).

AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (ARMD) is another progressive chronic disease that largely affects people >65 years old (63). Affected eyes have degeneration of photoreceptors and their underlying retinal pigment epithelium (RPE) in the macular area on the posterior pole of the eye. Clinically, ARMD is classified into a nonexudative (dry) and an exudative (wet) form. The dry form of ARMD is characterized by a slow and progressive degeneration of photoreceptors and the RPE (termed geographic atrophy). The wet form is a much more severe form of ARMD and usually leads to rapid loss of central vision from choroidal neovascularization and the resultant hemorrhage and scar formation. The incidence of the dry form of ARMD has been reported to be ~10%–30%, depending on the age. The wet form of ARMD has a much lower incidence but almost all affected eyes have various degrees of vision impairment.

The initial lesion of ARMD starts with the degeneration of RPE, although vision loss at later stages is a result of photoreceptor damage in the central retina

(38, 39, 114). No apoptotic RPE cells have been detected from eyes with the dry-form ARMD. This could be due to the slow progress of the dry-form ARMD over years to decades, compared with the quick turnover rate and phagocytosis of apoptotic cells in vivo. However, apoptotic RPE cells can be detected in choroidal neovascular membranes (47), and a variety of stimuli can induce in vitro-cultured RPE cells to undergo apoptosis (reviewed in 15), indicating that the apoptotic machinery is functional in this particular type of epithelial cell.

To date, the etiology of ARMD is still largely unknown, and available treatment is limited to only certain patients with the wet form of ARMD. There are strong epidemiological data suggesting that oxidative stress is a major contributing risk factor for ARMD (15), although a causal relationship has not yet been established. Consumption of diets that are rich in antioxidants is associated with a substantial decrease in risk (15, 112). In vitro studies show that oxidative stress induces apoptosis in RPE cells in a process whereby mitochondrial membrane potential declines, cytochrome *c* is released, and caspase-3-like activity increases (16, 17).

Antioxidants

Glutathione or its constituent amino acids protected RPE cells against oxidant-induced apoptosis. Plasma concentrations of glutathione are increased in association with increased dietary intake of antioxidants from fruits and vegetables (52). Thus, in principle, fruits and vegetables that are high in antioxidants could protect against ARMD indirectly by enhancing glutathione-dependent protection against oxidant-induced apoptosis. Alternatively, fruits and vegetables also contain dietary inducers of glutathione synthesis. Dimethylfumarate (DMF) is a non-nutritive compound found in fruits such as apples. Treatment of in vitro-cultured RPE cells with DMF or oltipraz increased the cellular glutathione and protected against oxidant-induced apoptosis (85, 119). Thus, in addition to antioxidants, dietary agents that induce cellular glutathione synthesis prevent oxidant-induced apoptosis in RPE cells.

Antioxidants most likely regulate the activation phase of oxidant-induced apoptosis. Oxidants can induce mitochondrial permeability transition and release of mitochondrial intermembrane proteins, such as cytochrome *c*, apoptosis inducing factor (AIF), and mitochondrial caspases. By shifting the cellular redox potential to a more reduced state, an increase of cellular glutathione inhibits the mitochondrial permeability transition. Oxidative stress can also up-regulate Fas/FasL expression on RPE cells (50), increasing the probability that apoptosis will be induced by this pathway. Glutathione was found to block the oxidant-induced Fas/FasL expression and apoptosis (50).

ATHEROSCLEROSIS

Atherosclerosis is characterized by progressive growth of plaques on the luminal surface of arteries. Plaques begin with the recruitment of inflammatory monocytes into the arterial cell wall (86), probably as the result of injury to the endothelial

cell barrier (100). The monocytes differentiate into tissue macrophages and, on ingestion of oxidized lipids, become foam cells. The lesion, known as a fatty streak at this stage, develops into an intimal thickening as smooth muscle cells accumulate within the intima of the artery. This process is accompanied by the further recruitment of inflammatory cells (T cells, macrophages, and mast cells) and degradation and deposition of basement membrane (vascular remodeling). The resulting atherosclerotic plaque can grow and eventually occlude flow through the affected artery. However, the clinical manifestations of atherosclerosis, such as myocardial infarction, stroke, abdominal aneurysms, and lower-limb ischemia usually arise from the sudden rupture of a plaque and the ensuing thrombosis (9, 40, 86, 128). Therefore, there has been intense interest in determining the factors that influence plaque stability.

Apoptotic smooth muscle cells, vascular endothelial cells, and foam cells have been observed in atherosclerotic plaques (10, 58, 106, 128). This apoptosis could be important both in plaque development and plaque rupture. Therefore, dietary factors affecting apoptosis could contribute to atherosclerosis and the associated life-threatening vascular occlusions.

An advanced atherosclerotic plaque consists of an acellular, lipid-rich core and a fibrous cap. The relative proportions of these two structures and the number of smooth muscle cells in the fibrous cap are the key determinants of plaque stability (40). Stable plaques tend to have substantial fibrous caps, whereas unstable, rupture-prone plaques have thin fibrous caps with relatively few viable smooth muscle cells. Vascular smooth muscle cells are essential for maintenance of the stabilizing fibrous cap structure (86). The net loss of smooth muscle cells in the cap region, resulting from an imbalance between proliferation and apoptosis of these cells, can contribute to the thinning and, thus, destabilization of the plaque (9). Foam cells, derived from macrophages that have engulfed large amounts of oxidized LDL particles, surround the lipid-rich core, and apoptotic foam cells tend to be found in rupture-prone shoulder regions of the plaques (40). The precise contribution of apoptosis in the development and destabilization of atherosclerotic plaques is unknown, but further research into this area will provide the basis for new therapeutic strategies based on inhibition of apoptosis to enhance plaque stability.

Antioxidants

Oxidized LDL particles contribute to the development of atherosclerotic plaques (9, 87). Macrophages take up oxidized LDL through scavenger receptors and a putative oxidized LDL receptor and become foam cells. Additionally, oxidized LDL can directly induce apoptosis (87) and can modulate inflammatory and thrombogenic mediators (96). Thus, prevention of LDL oxidation by antioxidants would be expected to inhibit disease progression. In addition, antioxidants are known to directly inhibit apoptosis in many cell lines (15). Epidemiological studies have demonstrated a strong inverse correlation between vitamin E intake and

atherosclerotic heart disease in men and women (99, 116). Vitamin E inhibits the ex vivo oxidation of serum LDLs (96). In randomized trials, vitamin E supplementation to patients with proven coronary heart disease either protected (118) or had no significant effect (98) on future occurrences of myocardial infarction. Additional studies are needed to determine whether protective effects involve inhibition of apoptosis or only occur upstream via inhibition of oxidized LDL formation.

Cholesterol

Hypercholesterolemia, age, gender, hypertension, smoking, and diabetes are major risk factors for atherosclerosis (128). Diets that are low in saturated fat and cholesterol can lower serum cholesterol and have proven beneficial for those at moderate atherosclerotic risk (53), whereas cholesterol-lowering drugs are commonly used to treat patients at high risk for coronary artery disease (53, 96). Blood cholesterol levels, particularly the ratio of LDL cholesterol to HDL cholesterol, affect plaque development by affecting the growth and nature of the lipid-rich core (72). Upon withdrawal of cholesterol and in the presence of high levels of HDL, cholesterol may actually be removed from the plaque (59, 96). In addition, in vitro studies show that 7-ketocholesterol, an oxidation product of cholesterol, can directly induce apoptosis in smooth muscle cells and macrophages in vitro (87). Thus, the well-established role of cholesterol in atherosclerosis may be mediated in part by induction of apoptosis by cholesterol oxidation products.

Zinc

Zinc has an antiapoptotic effect on a variety of cell types (18, 121). Zinc also functions as a membrane-stabilizing agent and as an antioxidant. Thus, zinc status may affect the development and progression of atherosclerosis. Hennig and coworkers have shown that the barrier function of cultured vascular endothelial cells is disrupted by zinc deficiency (46) and that zinc supplementation protects these cells from apoptosis induced by linoleic acid or by the inflammatory cytokine TNF (45). Whether these effects on endothelial cell integrity have a role in the development of atherosclerotic lesions is unclear, but may account for the presence of inflammatory infiltrates and apoptosis seen in these lesions (26).

IMMUNITY

Apoptosis plays a critical role in the development and maintenance of the immune system (103). T-cell development is dependent on both positive and negative selection in the thymus, during which thymocytes expressing inappropriate T-cell receptors are eliminated via apoptosis (104). As a defense against inadvertent activation of an immune response, T-cell receptor stimulation in the absence of a costimulatory signal induces apoptosis of mature T cells (103). Maturation of B cells within germinal centers of the spleen is dependent on removal of inferior or

self-reactive clones via apoptosis (109, 123). Cells mediating nonspecific immune responses, that is neutrophils and natural killer cells, are rapidly turned over. Antigen-specific T cells and B cells rapidly proliferate via clonal expansion upon infection. These clones are then deleted after the infection has been cleared (64). Thus, factors that influence apoptosis in immune cells could have profound effects on the ability to fight infections.

Zinc

Animals fed a zinc-deficient diet are highly susceptible to infection by a wide variety of pathogens (107, 135). Conversely, dietary zinc supplementation decreases both the rate of infection and the duration of infection in animal models. Zinc deficiency can develop in humans owing to malnutrition or malabsorption, or secondary to aging, obesity, or diabetes (18). Zinc appears to exert its effects on immunity at several different levels. Of particular importance, zinc has a well-documented antiapoptotic effect in many cell types, and this effect could be central to the changes in immune function observed during zinc deficiency and zinc supplementation.

In vitro studies show that zinc deficiency induces, whereas zinc supplementation inhibits, apoptosis in a wide variety of cell types (78, 121). In vivo, substantial atrophy of the thymus, the site of T-cell development, is also observed in zinc-deficient animals and humans (107). This atrophy is associated with increased thymocyte apoptosis. Similarly, the number of developing B cells in the bone marrow of zinc-deficient mice is significantly reduced. Thus, the deficits in cellular and humoral immunity caused by zinc deficiency may result in part from a decrease in the number of T cells and B cells caused by increased apoptosis. Skin lesions are also common in animals that are fed zinc-deficient diets and in humans with disorders in zinc absorption, and apoptosis probably plays a role in the formation of these lesions (84). Most of the research on zinc and apoptosis has involved models with profound changes in zinc; it remains unclear whether significant effects on apoptosis occur under more moderate changes in zinc availability.

FUTURE DIRECTIONS

The major conclusions from this review are that induction of apoptosis is likely to be an important mechanism in the cancer-preventive effects of diet and that inhibition of apoptosis may provide a mechanism by which diet can protect against certain disease processes associated with aging. However, improved methods are needed to measure apoptosis in vivo, and mechanistic details for dietary effects on apoptosis need to be further elucidated.

An additional feature emerges from this review that may have great practical importance, namely, that the assessment of whether a dietary component is beneficial cannot be based solely on in vitro studies. For example, an agent that activates

apoptosis in precancerous cells may be good for an individual at risk for tumor development but may not be so good for overall health if it also activates apoptosis in postmitotic cells and contributes to a degenerative disease. Conversely, an agent that inhibits apoptosis in postmitotic cells may not be useful in vivo if it also inhibits the normal physiologic mechanisms to eliminate precancerous cells. Thus, as knowledge accumulates on the effects of dietary factors on apoptosis, there is an increasing urgency for better methods and more studies to address dietary effects on apoptosis in vivo.

LITERATURE CITED

1. Adams JM, Cory S. 1998. The Bcl-2 protein family: arbiters of cell survival. *Science* 281:1322–26
2. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, et al. 1987. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* 262:5592–95
3. Ariga T, Jarvis WD, Yu RK. 1998. Role of sphingolipid-mediated cell death in neurodegenerative diseases. *J. Lipid Res.* 39:1–16
4. Ashkenazi A, Dixit VM. 1998. Death receptors: signaling and modulation. *Science* 281:1305–8
5. Bain JS, Shaw CA. 1997. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Res. Rev.* 25:335–58
6. Barnes S. 1997. The chemopreventive properties of soy isoflavonoids in animal models of breast cancer. *Breast Cancer Res. Treat.* 46:169–79
7. Belanger JT. 1998. Perillyl alcohol: applications in oncology. *Altern. Med. Rev.* 3:448–57
8. Bennett MR. 1999. Mechanisms of p53-induced apoptosis. *Biochem. Pharmacol.* 58:1089–95
9. Bennett MR. 1999. Apoptosis of vascular smooth muscle cells in vascular remodelling and atherosclerotic plaque rupture. *Cardiovasc. Res.* 41:361–68
10. Best PJ, Hasdai D, Sangiorgi G, Schwartz RS, Holmes DR Jr, et al. 1999. Apoptosis: basic concepts and implications in coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* 19:14–22
11. Block G, Patterson R, Subar A. 1992. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* 18:1–29
12. Bougnoux P. 1999. n-3 polyunsaturated fatty acids and cancer. *Curr. Opin. Clin. Nutr. Metab. Care* 2:121–60
13. Burke JR, Morgenlander JC. 1999. Update on Alzheimer's disease. *Postgrad. Med.* 106:85–96
14. Burke RE, Kholodilov NG. 1998. Programmed cell death: does it play a role in Parkinson's disease? *Ann. Neurol.* 44 (Suppl. 1):S126–31
15. Cai J, Nelson KC, Wu M, Sternberg P Jr, Jones DP. 1999. Oxidative damage and protection of the RPE. *Prog. Retin. Eye Res.* 19:205–21
16. Cai JY, Wu M, Nelson KC, Sternberg P Jr, Jones DP. 1999. Oxidant-induced apoptosis in cultured human retinal pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 40:959–66
17. Cai J, Yang J, Jones DP. 1998. Mitochondrial control of apoptosis: the role of cytochrome c. *Biochim. Biophys. Acta* 1366:139–50
18. Chai F, Truong-Tran AQ, Ho LH, Zalewski

PD. 1999. Regulation of caspase activation and apoptosis by cellular zinc fluxes and zinc deprivation: a review. *Immunol. Cell Biol.* 77:272–78

19. Chang WL, Chapkin RS, Lupton JR. 1998. Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. *J. Nutr.* 128:491–97
20. Chen Y-R, Wang W, Kong A-NT, Tan T-H. 1998. Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. *J. Biol. Chem.* 273:1769–75
21. Cohen GM. 1997. Caspases: the executioners of apoptosis. *Biochem. J.* 326:1–16
22. Constantinou AI, Kamath N, Murley JS. 1998. Genistein inactivates bcl-2, delays the G21/M phase of the cell cycle, and induces apoptosis of human breast adenocarcinoma MCF-7 cells. *Eur. J. Cancer* 34:1927–34
23. Crowell PL. 1999. Prevention and therapy of cancer by dietary monoterpenes. *J. Nutr.* 129:775–78S
24. Davis JN, Singh B, Bhuiyan M, Sarkar FH. 1998. Genistein-induced upregulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. *Nutr. Cancer* 32:123–31
25. DeLong MJ. 1998. Apoptosis: a modulator of cellular homeostasis and disease states. *Ann. NY Acad. Sci.* 842:82–90
26. Dimmeler S, Hermann C, Zeiher AM. 1998. Apoptosis of endothelial cells: contribution to the pathophysiology of atherosclerosis? *Eur. Cytokine Netw.* 9: 697–98
27. Dragunow M, MacGibbon GA, Lawlor P, Butterworth N, Connor B, et al. 1997. Apoptosis, neurotrophic factors and neurodegeneration. *Rev. Neurosci.* 8:223–65
28. Du Y, Dodel RC, Bales KR, Jemmerson R, Hamilton-Byrd E, Paul SM. 1997. Involvement of a caspase-3-like cysteine protease in 1-methyl-4-phenylpyridinium-mediated apoptosis of cultured cerebellar granule neurons. *J. Neurochem.* 69:1382–88
29. Duan W, Zhang Z, Gash DM, Mattson MP. 1999. Participation of prostate apoptosis response-4 in degeneration of dopaminergic neurons in models of Parkinson's disease. *Ann. Neurol.* 46:587–97
30. Elegbede JA, Maltzman TH, Elson CE, Gould MN. 1993. Effects of anticarcinogenic monoterpenes on phase II hepatic metabolizing enzymes. *Carcinogenesis* 14:1221–23
31. Evan G, Littlewood T. 1998. A matter of life and cell death. *Science* 281:1317–22
32. Fan YY, Zhang J, Barhoumi R, Burghardt RC, Turner ND, et al. 1999. Antagonism of CD95 signaling blocks butyrate induction of apoptosis in young adult mouse colonic cells. *Am. J. Physiol.* 277:C310–19
33. Friedlander RM, Yuan J. 1998. ICE, neuronal apoptosis and neurodegeneration. *Cell Death Differ.* 5:823–31
34. Fritz WA, Coward L, Wang J, Lamartiniere CA. 1998. Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. *Carcinogenesis* 19:2151–58
35. Gelb MH, Scholten JD, Sebolt-Leopold JS. 1998. Protein prenylation: from discovery to prospects for cancer treatment. *Curr. Opin. Chem. Biol.* 2:40–48
36. Ghadge GD, Lee JP, Bindokas VP, Jordan J, Ma L, et al. 1997. Mutant superoxide dismutase-1-linked familial amyotrophic lateral sclerosis: molecular mechanisms of neuronal death and protection. *J. Neurosci.* 17:8756–66
37. Gould MN. 1997. Cancer chemoprevention and therapy by monoterpenes. *Environ. Health Perspect.* 105(Suppl. 4):977–79
38. Green WR, Key SN III. 1977. Senile macular degeneration: a histopathologic study. *Trans. Am. Ophthalmol. Soc.* 75:180–254
39. Green WR, Wilson DJ. 1986. Choroidal neovascularization. *Ophthalmology* 93: 1169–76

40. Gutstein DE, Fuster V. 1999. Pathophysiology and clinical significance of atherosclerotic plaque rupture. *Cardiovasc. Res.* 41:323–33
41. Haufel T, Dormann S, Hanusch J, Schwieger A, Bauer G. 1999. Three distinct roles for TGF-beta during intercellular induction of apoptosis: a review. *Anticancer Res.* 19:105–11
42. Hecht SS. 1999. Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. *J. Nutr.* 129:768–74S
43. Heerdt BG, Houston MA, Anthony GM, Augenlicht LH. 1999. Initiation of growth arrest and apoptosis of MCF-7 mammary carcinoma cells by tributyrin, a triglyceride analogue of the short-chain fatty acid butyrate, is associated with mitochondrial activity. *Cancer Res.* 59:1584–91
44. Heerdt BG, Houston MA, Augenlicht LH. 1997. Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. *Cell Growth Diff.* 8:523–32
45. Hennig B, Meerarani P, Ramadass P, Toborek M, Malecki A, et al. 1999. Zinc nutrition and apoptosis of vascular endothelial cells: implications in atherosclerosis. *Nutrition* 15:744–48
46. Hennig B, Wang Y, Ramasamy S, McClain CJ. 1992. Zinc deficiency alters barrier function of cultured porcine endothelial cells. *J. Nutr.* 122:1242–47
47. Hinton DR, He S, Lopez PF. 1998. Apoptosis in surgically excised choroidal neovascular membranes in age-related macular degeneration. *Arch. Ophthalmol.* 116:203–9
48. Iacovitti L, Stull ND, Mishizen A. 1999. Neurotransmitters, KCl and antioxidants rescue striatal neurons from apoptotic cell death in culture. *Brain Res.* 816:276–85
49. Imai Y, Kimura T, Murakami A, Yajima N, Sakamaki K, Yonehara S. 1999. The CED-4 homologous protein FLASH is involved in Fas-mediated activation of caspase-8 during apoptosis. *Nature* 398:777–85
50. Jiang S, Wu MH, Sternberg P Jr, Jones DP. 2000. Fas mediates apoptosis and oxidant-induced cell death in cultured human retinal pigment epithelial cells. *Invest. Opthalmol. Vis. Sci.* 41:645–55
51. Johnston CS, Meyer CG, Srilakshmi JC. 1993. Vitamin C elevates red blood cell glutathione in healthy adults. *Am. J. Clin. Nutr.* 58:103–5
52. Jones DP, Brown LAS, Sternberg P Jr. 1995. Variability in glutathoine-dependent detoxication in vivo and its relevance to detoxication of chemical mixtures. *Toxicology* 105:267–74
53. Jukema JW, Simoons ML. 1999. Treatment and prevention of coronary heart disease by lowering serum cholesterol levels; from the pioneer work of C.D. de Langen to the third "Dutch Consensus on Cholesterol." *Acta Cardiol.* 54:163–68
54. Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26:239–57
55. Kirlin WG, Cai J, DeLong MJ, Patten EJ, Jones DP. 1999. Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. *J. Nutr.* 129:1827–35
56. Kirlin WG, Cai J, Thompson SA, Diaz D, Kavanagh TJ, Jones DP. 1999. Glutathione redox potential in response to differentiation and enzyme inducers. *Free Rad. Biol. Med.* 27(11–12):1208–18
57. Klevenyi P, Andreassen O, Ferrante RJ, Schleicher JR Jr, Friedlander RM, Beal MF. 1999. Transgenic mice expressing a dominant negative mutant interleukin-1beta converting enzyme show resistance to MPTP neurotoxicity. *NeuroReport* 10:635–38
58. Kockx MM. 1998. Apoptosis in the atherosclerotic plaque. *Arterioscler. Thromb. Vasc. Biol.* 18:1519–22
59. Kockx MM, De Meyer GR, Burssens N,

Knaapen MW, Bult H, Herman AG. 1998. Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal. *Circ. Res.* 83:378–87
60. Kolonel LN. 1997. Fat and cancer: the epidemiologic evidence in perspective. *Adv. Exp. Med. Biol.* 422:1–19
61. Lane DP. 1992. p53, guardian of the genome. *Nature* 358:15–16
62. Latham P, Lund EK, Johnson IT. 1999. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. *Carcinogenesis* 20:645–50
63. Leibowitz HM, Krueger DE, Maunder LR, Milton RC, Kini MM, et al. 1980. The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973–1975. *Surv. Ophthalmol.* 24(Suppl.):335–610
64. Leonardo M, Chan KM, Hornung F, McFarland H, Siegel R, et al. 1999. Mature T lymphocyte apoptosis-immune regulation in a dynamic and unpredictable antigenic environment. *Annu. Rev. Immunol.* 17:221–53
65. Leppa S, Bohmann D. 1999. Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* 18:6158–62
66. Levi F. 1999. Cancer prevention: epidemiology and perspectives. *Eur. J. Cancer* 35:1046–58
67. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad ES, et al. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase 9 complex initiates an apoptotic protease cascade. *Cell* 91:479–89
68. Li D, Saldeen T, Mehta JL. 1999. Gamma-tocopherol decreases ox-LDL-mediated activation of nuclear factor-kappaB and apoptosis in human coronary artery endothelial cells. *Biochem. Biophys. Res. Commun.* 259:157–61
69. Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. 1999. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. *Oncogene* 18:3166–72
70. Lian F, Li Y, Bhuiyan M, Sarkar FH. 1999. p53-independent apoptosis induced by genistein in lung cancer cells. *Nutr. Cancer* 33:125–31
71. Lipkin M, Reddy B, Newmark H, Lamprecht SA. 1999. Dietary factors in human colorectal cancer. *Annu. Rev. Nutr.* 19:545–86
72. Loree HM, Tobias BJ, Gibson LJ, Kamm RD, Small DM, Lee RT. 1994. Mechanical properties of model atherosclerotic lesion lipid pools. *Arterioscler. Thromb.* 14:230–34
73. Luo X, Imawati B, Zou H, Clive S, Wang X. 1998. Bid, a Bcl-2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 94:481–90
74. Mancini M, Anderson BO, Caldwell E, Sedghinasab M, Paty PB, Hockenbery DM. 1997. Mitochondrial proliferation and paradoxical membrane depolarization during terminal differentiation and apoptosis in a human colon carcinoma cell line. *J. Cell. Biol.* 138:449–69
75. Marsden CD, Olanow CW. 1998. The causes of Parkinson's disease are being unraveled and rational neuroprotective therapy is close to reality. *Ann. Neurol.* 44(Suppl. 1):S189–96
76. Mathers JC. 1998. Nutrient regulation of intestinal proliferation and apoptosis. *Proc. Nutr. Soc.* 57:219–23
77. Mayeux R, Sano M. 1999. Drug therapy: treatment of Alzheimer's disease. *N. Engl. J. Med.* 341:1670–79
78. McCabe MJ Jr, Jiang SA, Orrenius S. 1993. Chelation of intracellular zinc triggers apoptosis in mature thymocytes. *Lab. Invest.* 69:101–10

79. McCabe MJ Jr, Orrenius S. 1993. Genistein induces apoptosis in immature human thymocytes by inhibiting topoisomerase-II. *Biochem. Biophys. Res. Commun.* 194:944–50
80. Medina V, Edmonds B, Young GP, James R, Appleton S, Zalewski PD. 1997. Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependent pathway. *Cancer Res.* 57:3697–707
81. Merrill AH Jr, Schmelz EM, Wang E, Dillehay DL, Rice LG, et al. 1997. Importance of sphingolipids and inhibitors of sphingolipid metabolism as components of animal diets. *J. Nutr.* 127:830S–33
82. Michel PP, Lambeng N, Ruberg M. 1999. Neuropharmocologic aspects of apoptosis: significance for neurodegenerative diseases. *Clin. Neuropharmacol.* 22:137–50
83. Mills JJ, Chari RS, Poyer IJ, Gould MN, Jirtle RL. 1995. Induction of apoptosis in liver tumors by the monoterpene perillyl alcohol. *Cancer Res.* 55:979–83
84. Mori H, Matsumoto Y, Tamada Y, Ohashi M. 1996. Apoptotic cell death in formation of vesicular skin lesions in patients with acquired zinc deficiency. *J. Cutaneous Pathol.* 23:359–63
85. Nelson KC, Carlson JL, Newman ML, Sternberg P Jr., Jones DP, et al. 1999. Effect of dietary inducer dimethylfumarate on glutathione in cultured human retinal pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 40:1927–35
86. Newby AC, Zaltsman AB. 1999. Fibrous cap formation and destruction: the critical importance of vascular smooth muscle cell proliferation, migration and matrix formation. *Cardiovasc. Res.* 41:345–60
87. Nishio E, Arimura S, Watanabe Y. 1996. Oxidized LDL induces apoptosis in cultured smooth muscle cells, macrophages and fibroblasts. *Arterioscler. Thromb. Vasc. Biol.* 16:416–18
88. Oates AJ, Schumaker LM, Jenkins SB, Pearce AA, DaCosta SA, et al. 1998. The mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGR2R), a putative breast tumor suppressor gene. *Breast Cancer Res. Treat.* 47:269–81
89. Onozawa M, Fukuda K, Ohtani M, Akaza H, Sugimura T, Wakabayashi K. 1998. Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. *Jpn. J. Clin. Oncol.* 28:360–63
90. Onozawa M, Kawamori T, Baba M, Fukuda K, Toda T, et al. 1999. Effects of a soybean isoflavone mixture on carcinogenesis in prostate and seminal vesicles of F344 rats. *Jpn. J. Cancer Res.* 90:393–98
91. Parodi PW. 1997. Cows' milk fat components as potential anticarcinogenic agents. *J. Nutr.* 127:1055–60
92. Pereira C, Santos MS, Oliveira C. 1999. Involvement of oxidative stress on the impairment of energy metabolism induced by A beta peptides on PC12 cells: protection by antioxidants. *Neurobiol. Dis.* 6:209–19
93. Pillot T, Drouet B, Queille S, Labeur C, Vandekerchkhove J, et al. 1999. The nonfibrillar amyloid beta-peptide induces apoptotic neuronal cell death: involvement of its C-terminal fusogenic domain. *J. Neurochem.* 73:1626–34
94. Prasad KN, Cole WC, Kumar B. 1999. Multiple antioxidants in the prevention and treatment of Parkinson's disease. *J. Am. Coll. Nutr.* 18:413–23
95. Przedborski S, Jackson-Lewis V. 1998. Mechanisms of MPTP toxicity. *Mov. Disord.* 13(Suppl. 1):35–38
96. Rabbani R, Topol EJ. 1999. Strategies to achieve coronary arterial plaque stabilization. *Cardiovasc. Res.* 41:402–17
97. Rao CV, Rivenson A, Kelloff GJ, Reddy BS. 1995. Chemoprevention of azoxymethane-induced colon cancer by

ascorbylpalmitate, carbenoxolone, dimethylfumarate and p-methoxyphenol in male F344 rats. *Anticancer Res.* 15:1199–204

98. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, et al. 1997. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet* 349:1715–20
99. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. 1993. Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* 328:1450–56
100. Ross R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362:801–9
101. Sakamoto K, Lawson LD, Milner JA. 1997. Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. *Nutr. Cancer* 29:152–56
102. Samali A, Zhivotovsky B, Jones D, Nagata S, Orrenius S. 1999. Apoptosis: cell death defined by caspase activation. *Cell Death Differ.* 6:495-96
103. Scaffidi C, Kirchhoff S, Krammer PH, Peter ME. 1999. Apoptosis signaling in lymphocytes. *Curr. Opin. Immunol.* 11:277–85
104. Sebzda E, Mariathasan S, Ohteki T, Jones R, Bachmann MF, Ohashi PS. 1999. Selection of the T cell repertoire. *Annu. Rev. Immunol.* 17:829–74
105. Setchell KDR, Cassidy A. 1999. Dietary isoflavones: biological effects and relevance to human health. *J. Nutr.* 129:758–67S
106. Shah PK. 1999. Plaque disruption and thrombosis. Potential role of inflammation and infection. *Cardiol. Clin.* 17:271–81
107. Shankar AH, Prasad AS. 1998. Zinc and immune function: the biological basis of altered resistance to infection. *Am. J. Clin. Nutr.* 68:447–63S
108. Shao ZM, Alpaugh ML, Fontana JA, Barsky SH. 1998. Genistein inhibits proliferation similarly in estrogen receptor-positive and negative human breast carcinoma cell lines characterized by p21WAF1/CIP1 induction, G2/M arrest, and apoptosis. *J. Cell. Biochem.* 69:44–54
109. Shokat KM, Goodnow CC. 1995. Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature* 375:334–38
110. Shoulson I. 1998. DATATOP: a decade of neuroprotective inquiry. Parkinson study group. Deprenyl and tocopherol antioxidative therapy of Parkinsonism. *Ann. Neurol.* 44(3 Suppl. 1):S160–66
111. Singh A, Shukla Y. 1998. Antitumor activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. *Cancer Lett.* 131:209–14
112. Snodderly DM. 1995. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.* 62(6 Suppl.):1448–61S
113. Spiegel S, Merrill AH Jr. 1996. Sphingolipid metabolism and cell growth regulation. *FASEB J.* 10:1388–97
114. Spraul CW, Lang GE, Grossniklaus HE. 1996. Morphometric analysis of the choroid, Bruch's membrane and retinal pigment epithelium in eyes with age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* 37:2724–35
115. Srivastava SK, Hu X, Xia H, Zaren HA, Chatterjee ML, et al. 1997. Mechanism of differential efficacy of garlic organosulfides in preventing benzo(a)pyrene-induced cancer in mice. *Cancer Lett.* 118:61–67
116. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. 1993. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* 328:1444–49
117. Stefanis L, Burke RE, Greene LA. 1997.

Apoptosis in neurodegenerative disorders. *Curr. Opin. Neurol.* 10:299–305
118. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. 1996. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 347:781–86
119. Sternberg P Jr, Nelson KC, Jones DP. 1999. Oltipraz increases glutathione in human retinal epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 40:S160
120. Suhara T, Fukuo K, Sugimoto T, Morimoto S, Nakahashi T, et al. 1998. Hydrogen peroxide induces up-regulation of Fas in human endothelial cells. *J. Immunol.* 160:4042–47
121. Sunderman FW Jr. 1995. The influence of zinc on apoptosis. *Ann. Clin. Lab. Sci.* 25:134–42
122. Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, et al. 1999. Parkinson disease in twins: an etiologic study. *J. Am. Med. Assoc.* 281:341–46
123. Tarlington DM, Smith KG. 1997. Apoptosis and the B cell response to antigen. *Int. Rev. Immunol.* 15:53–71
124. Tatton WG, Chalmers-Redman RME. 1998. Mitochondria in neurodegenerative apoptosis: an opportunity for therapy? *Ann. Neurol.* 44(3 Suppl. 1):S134–41
125. Tatton WG, Olanow CW. 1999. Apoptosis in neurodegenerative diseases: the role of mitochondria. *Biochim. Biophys. Acta* 1410:195–213
126. Thompson CB. 1995. Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456–62
127. Vanden Heuvel JP. 1999. Peroxisome proliferator-activated receptors: a critical link among fatty acids, gene expression and carcinogenesis. *J. Nutr.* 129:575–80S
128. van der Wal AC, Becker AE. 1999. Atherosclerotic plaque rupture: pathologic basis of plaque stability and instability. *Cardiovasc. Res.* 41:334–44
129. Vesper H, Schmelz EM, Nikolova-Karakashian MN, Dillehay DL, Lynch DV, Merrill AH Jr. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *J. Nutr.* 129:1239–50
130. Winkler BS, Boulton ME, Gottsch JD, Sternberg P Jr. 1999. Oxidative damage and age-related macular degeneration. *Mol. Vis.* 5:32–43
131. Wright SC, Zhong J, Larrick JW. 1994. Inhibition of apoptosis as a mechanism of tumor promotion. *FASEB J.* 8:654–60
132. Yang L, Matthews RT, Schulz JB, Klockgether T, Liao AW, et al. 1998. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyride neurotoxicity is attenuated in mice overexpressing Bcl-2. *J. Neurosci.* 18:8145–52
133. Yatin SM, Aksenov M, Butterfield DA. 1999. The antioxidant vitamin E modulates amyloid beta-peptide-induced creatine kinase activity inhibition and increased protein oxidation: implications for the free radical hypothesis of Alzheimer's disease. *Neurochem. Res.* 24: 427–35
134. Yu R, Mandlekar S, Harvey KJ, Ucker DS, Kong A-NT. 1998. Chemopreventive isothiocyanates induce apoptosis and caspase-3-like activity. *Cancer Res.* 58:402–8
135. Zalewski PD. 1996. Zinc and immunity: implications for growth survival and function of lymphoid cells. *J. Nutr. Immunol.* 4:39–101
136. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. 1999. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. *J. Nutr.* 129:1628–35
137. Zhou JR, Mukherjee P, Gugger ET, Tanaka T, Blackburn GL, Clinton SK. 1998. Inhibition of murine bladder tumorigenesis by soy isoflavones via alterations in the cell cycle, apoptosis, and angiogenesis. *Cancer Res.* 58:5231–38