Emerging Clinical Science of Bifunctional Support for Detoxification

By DeAnn J. Liska, Ph.D. and Jeffrey S. Bland, Ph.D.

An enormous amount of research literature associating toxin exposure to disease has been published, and current estimates suggest that between $568 and $793 billion dollars is spent in the U.S. and Canada every year on toxicity-related diseases.(Muir and Zegarac, 2001) Exposure to toxins -- such as heavy metals, pesticides, industrial compounds and pollutants -- is a causative factor for many types of cancers, Parkinson's disease, conditions such as chronic fatigue syndrome (CFS) and multiple chemical sensitivities (MCS), and is suggested to affect diverse conditions such as atherosclerosis and diabetes.(Sherer et al., 2002; Racciatti et al., 2001; Olmstead, 2000; Silkworth and Brown, 1996) Cancer statistics alone are alarming; environment may account for as much as 80% of cancer cases.(Lichtenstein et al., 2000) Cancer is the third leading cause of death in children.(CDC, 2000) In particular, the incidence of non-Hodgkin's lymphoma and brain cancer in children have increased 30% and 21%, respectively, between 1973 and 1997, and strong associations exist between these cancers and exposure to environmental toxins like organochlorinated pesticides.(Buckley et al., 2000; Daniels et al., 1997; Meinert et al., 2000; Rothman et al., 1997; Webster et al., 2002)

The question for preventive medicine is: How do we protect ourselves and our patients from the damage toxins can produce in order to promote optimal health and longevity? Certainly, decreasing the amount of toxins in the environment, and minimizing exposure to toxins are key in any strategy to decrease risk of myriad conditions. Yet, these strategies alone are not enough. We can't escape toxin exposure in today's world. A 2001 pilot study looking at residential toxin exposure detected the presence of 33 different carcinogens implicated in breast cancer in house dust, and 24 different compounds in air.(Rudel et al., 2001) Investigations on tissue samples in humans show that we maintain a level of toxin contamination within our bodies on a regular basis. For example, studies in 1995 showed that several toxins, including naphthols and chlorinated polyphenols, were found in tissues from at least 64% of the 1000 people studied, with the majority of these toxins present in over 80% of the tissues sampled.(Levin, 1999) These data indicate that we are all carrying a toxic load within our bodies due to a lifetime of exposure, and this toxic burden can accumulate so the body tissues are exposed to much higher doses than the environmental concentrations would suggest are present.

We simply cannot remove ourselves from all exposures to toxins. What we can do to provide protection from the influence these toxins can have on our health by promoting the endogenous systems that protect the body from their effects. The primary system that performs this task is the detoxification, or biotransformation system of enzymes, which includes Phase I bioactivation, and the Phase II conjugation. And, since these enzyme systems require many key nutrients to function optimally, phytonutrients can play a major role in promoting healthy detoxification and protection from toxicity.

Detoxification: One of the body's major defenses to environmental stress

The detoxification system is the main means by which lipid-soluble toxins are removed from the body. By far, the majority of toxins and drugs are lipid-soluble and, therefore, to be efficiently and safely removed via urine must be converted to a non-toxic water-soluble molecule during metabolism. This process occurs in two phases that work in concert with each other: Phase I adds an attachment site (bioactivation) to the toxin; and, Phase II attaches a water-soluble moiety to the toxin (conjugation).(reviewed in Liska, 1998) The Phase I enzymes bioactivate the toxin by adding a hydroxyl group to the lipid-soluble toxin. In doing so, Phase I uses the reduced form of nicotinamine adenosine dinucleotide (NADH) as a cofactor. Many dietary ingredients support Phase I reactions, including vitamin B3 (niacin), which is required for generation of NADH. In addition, the bioactivation reaction often also generates reactive oxygen species directly as a spin-off product, since it uses oxygen; therefore, dietary antioxidants can help protect tissue from damage that may occur by this reaction.

One of the consequences of Phase I bioactivation is that the product, called the reactive intermediate, is quite often more reactive -- and potentially more toxic -- than the parent molecule. Therefore, it is important that this molecule be converted to a non-toxic, water-soluble molecule as soon as possible. Conjugation of the reactive intermediate to a water-soluble molecule is accomplished by the Phase II conjugation reactions, which include glucuronidation, sulfation, glutathione conjugation, amino acid conjugation, methylation, and acetylation. These Phase II reactions require a replenishable source of cofactors-such as sulfate in the case of sulfation or specific amino acids the case of amino acid conjugation -- since these cofactors are attached to the toxin and then lost through excretion. The Phase II reactions not only require a steady source of cofactors, but also use large amounts of energy in the form of adenosine triphosphate (ATP). Therefore, ATP must be adequately replenished for optimal, balanced detoxification.

The detoxification system is quite complex. It occurs mainly in the liver, although the small intestine is very important in removing toxins during first-pass

metabolism. In fact, about 25% of detoxification and removal of toxins occurs in the intestine, which is significant not only in the amount of activity but also because once toxins are deactivated in the intestines they never enter the body. So, intestinal health is also important for optimal detoxification. However, it is important to note that all cells in the body have some of the Phase I and Phase II enzymes with which to perform detoxification and provide protection from toxins.

Phase I Bioactivation

Requires NADH (produced from niacin)

Requires oxygen, and often results in a more reactive toxin

Generates reactive oxygen species as spin-off products

Antioxidant support is crucial to protect from reactive oxygen species and reactive intermediates

Phase II Conjugation

Conjugation reactions include glucuronidation, sulfation, methylation, amino acid conjugation, glutathione conjugation, and acetylation

Requires a constant, replenishing source of the cofactors for these conjugation reactions, including taurine, glycine, glutamine, sulfate reserves, glutathione, methylation cofactors, and healthy serum glucose levels

Requires large amounts of energy (ATP)

Conjugation enzymes can be induced with bifunctional modulators

---

Clinical Considerations for Programs to Support Optimal, Balanced Biotransformation

Decrease total load and exposure to toxins.

Provide low-allergy potential protein and high-energy, healthy fats to support amino acid needs and energy production for detoxification, while maintaining low toxin load and healthy blood glucose.

Provide bifunctional modulators to balance Phase I and Phase II activities.

Provide a complete, full spectrum nutrient support for Phase II conjugation reactions. In particular, provide strong support for key methylation pathways.

Support healthy liver function, especially with strong antioxidant protection to protect from Phase I generated reactive oxygen species and promote detoxification of toxic metals.

Support healthy digestion and excretion.

Clinical programs to promote healthy balanced detoxification should take into consideration several factors. Adequate nutriture for overall support of biotransformation and excretion is key, as well as provision of the full spectrum of Phase II cofactors. In addition, emerging research suggests that many phytonutrients that are associated with lower risk of cancers may exert their protective influence because they are bifunctional modulators of the detoxification system. A brief review of these clinical considerations is provided below.

Decrease total load and exposure to toxicants.

Decreasing exposure to toxins is extremely important to promote healthy detoxification and lower risk of toxin-associated disease. Airborne toxins are of particular concern since, by entering through nasal passages, they can bypass the blood-brain barrier and travel through the olfactory tract directly to the brain. Removal of toxins from the environment is a straightforward concept, but, since it usually involves lifestyle changes, can be difficult to fully implement. In addition, toxin exposure can occur at work as well as at home, so all environments should be considered.

Macronutrients to Support Detoxification

Several studies have shown that water or juice fasting can be detrimental to the body's ability to support detoxification and excretion of toxins. These fasting programs result in catabolism of muscle over fat, since the body needs a continual source of amino acids for production of new enzymes and proteins. When amino acids are not available from the diet, the body starts breaking down its own protein

reserves -- muscle. Water fasting is also of concern since it means a decreased intake of the necessary Phase II cofactors, which may lead to unbalanced detoxification. This has been demonstrated in animal models, in which water fasting results in decreased glutathione levels. Animals on water fasting programs have been shown to have an enhanced susceptibility to toxicity symptoms after a toxin exposure.(Fry et al., 1999) Fasting also leads to an over-induction of some Phase I enzymes, promoting even more unbalanced detoxification.(Lall et al., 1999) Detoxification is an energy-requiring process that puts a metabolic burden on the body, so instead of decreasing nutrient support, a focused, high-impact source of nutrients is essential.

A high-quality, complete source of protein should be low-allergy-potential in order to decrease the body's burden of inflammation and potential allergen toxins. High-quality protein is a good source of methionine, cysteine, glutamine, and glycine in a form that provides high absorption; these amino acids can be used to generate sulfation, glutathione and amino acid conjugation cofactors. A high-quality protein also may benefit those with toxic burdens of mercury, since mercury exposure is associated with depletion of the specific amino acids that are precursors to neurotransmitters. Cell culture studies have shown that mercury inhibits uptake and release of neurotransmitters such as dopamine, norepinephrine, and serotonin.(Quig, 1998) Methionine is also a component of the homocysteine cycle, which provides S-adenosylmethionine, the cofactor for Phase II methylation.

Support for energy production is also vital during detoxification; therefore, adequate intake of high energy-supportive nutrients are essential.(Lall et al., 1999) Fats can be problematic, since many people consume too many of the wrong kind. Moreover, individuals with toxicity-related conditions may have altered intestinal permeability (leaky gut) as one of the consequences of toxic exposure and, therefore, may not efficiently absorb nutrients like long-chain fats through the intestinal tract. Provision of a highly bioavailable source of fats that can be used directly to support energy production is beneficial. The medium chain triglycerides (MCTs) are fats that fit this profile.(DeGaetano et al., 1994) MCTs are not absorbed like long-chain fats, but are quickly metabolized in the small intestine and can be absorbed without the presence of bile. Moreover, the small intestine has greater capacity to absorb MCTs, and MCTs have been shown to support patients with malabsorption syndrome. MCTs have been shown to prevent early alcohol-induced liver injury in animals, possibly due to their ability to inhibit generation of reactive oxygen species.(Kono et al., 2000) Interestingly, olive oil, in contrast to sunflower, corn, or fish oil, was found to be protective against chemically-induced fibrosis in rats,(Szende et al., 1994) suggesting it may also be a good source of fat for a detoxification program.

Bifunctional Support for Detoxification: Achieving Balance

As mentioned above, Phase I bioactivation is necessary to provide an active site for attachment of the water-soluble group; however, Phase I bioactivation, by its name, "activates" the toxin to a more reactive compound. This double-edged sword of Phase I means that some activity is essential, but too much activity can result in generation of these reactive intermediates too quickly for Phase II to neutralize them into non-toxic, excretable molecules. Some phytonutrients support Phase I activity, such as indole-3-carbinol from broccoli, which provides a modest support for the Phase I enzymes. Over-activation of Phase I is of concern, however, and is associated with high, continuous levels of toxin exposure. Many toxins are extremely effective at over-inducing Phase I enzymes. For example, smoking, heterocyclic amines formed on charbroiled beef, and dioxin have all been shown to over-induce the Phase I CYP1A enzymes; and, low doses of these compounds appear to induce CYP1A much more effectively than the modest support provided by the phytonutrients associated with cancer protection, such as catechins and indole-3-carbinol.(McDanell et al., 1992; Vanden Heuvel et al., 1994; Kall and Clausen, 1995)

As can be elucidated from its name, a compound that provides bifuctional modulation for detoxification is one that supports healthy, optimal activity of both Phase I and Phase II. In the case of Phase II, healthy, optimal activity is associated with induction of the enzymes, thereby providing for higher activity. Support for healthy, optimal Phase I requires managing a balanced level of Phase I enzymes. Bifunctional modulators often are capable of inhibiting the induction of Phase I enzymes by toxins, without inhibiting Phase I entirely.

Since there are many Phase II enzymes, an effective bifunctional modulator will promote several of these Phase II activities at the same time. Many of the bifunctional modulators also promote optimal balance by their ability to act as antioxidants and quench reactive oxygen species from Phase I reactions. Therefore, bifunctional modulators support optimal detoxification balance by modulating Phase I activities, inducing several Phase II activities, and minimizing damage by reactive molecules. Several phytonutrients that are associated with protection from toxin damage do so by acting as bifunctional modulators; these include ellagic acid, found in pomegranate and many berries, catechins from green tea and grapes, and the glucosinolates found in crucifers, such as watercress and broccoli.

Ellagic Acid

In animal studies, ellagic acid has been shown to significantly reduce chemically-induced lung and liver tumorigenesis, protect from carbon tetrachloride liver damage, enhance glutathione production and decrease lipid peroxidation.(Khanduja

et al., 1999; Singh et al., 1999; Ahn et al., 1996) Ellagic acid may also act directly against some metal toxicity, such as nickel, by chelating the metal and promoting its excretion, providing protection from liver damage and oxidative stress.(Ahmed et al., 1999) Ellagic acid is a bifunctional modulator that promotes balanced detoxification by several mechanisms: it induces production of glutathione-S-transferases and other Phase II activities at the gene level, it modulates Phase I activities so these enzymes are not over-induced, and it can bind directly to some toxic substances, rendering them non-toxic and promoting their excretion. For example, ellagic acid can inhibit the induction of Phase I CYP1A by the mutagen benzo[a]pyrene, but does not directly inhibit constitutive, necessary activity of CYP1A.(Barch et al., 1996) Ellagic acid can also bind directly to DNA, protecting the DNA from carcinogenic mutations.

Catechins

A large body of literature studying the health benefits of catechins has accumulated. These data indicate that the catechins are bifunctional modulators that provide many beneficial activities, including induction of Phase II glucuronidation and glutathione conjugation enzymes. Prospective animal experiments have shown that green tea catechins possess anticarcinogenic and antimutagenic potential.(Ahmad and Muktar, 1999; McKay and Blumberg, 2002) These compounds are strong antioxidants, data suggest that consumption of catechins may be associated with lower incidence of Parkinson's disease and many types of cancer.(Checkoway et al., 2002; Ross et al., 2000) These activities have prompted the National Cancer Institute to investigate the potential of green tea extract containing catechins as a chemotherapeautic agent.(Steele et al., 2000)

Interestingly, catechins have been shown to induce some Phase I activities;(Abbas et al., 1994; Xu et al., 1996) however, data also suggests that catechins selectively inhibit some Phase I activities as well.(Dashwood et al., 1999) A recent cell culture study showed that catechins inhibited the over-induction of Phase I activities by a toxic substance, but were able to moderately induce the Phase I activity themselves when the carcinogen was not present.(Williams et al., 2000) This ability to modify levels of Phase I -- promoting a moderate induction and inhibiting an over-induction -- may account for some of the beneficial activities of catechins. This study also indicated that the full spectrum of catechins was necessary for this effect, and different catechins provide differential Phase I antagonist and agonist functions.

The strong antioxidant activity of catechins also provides ability of catechins to bind to the reactive intermediates produced by Phase I that are not immediately conjugated by a Phase II reactions, which is another reason catechins may promote balance. Green tea catechins have also been shown to promote healthy microflora,

pH, and bowel function(Goto et al., 1999), which may further support detoxification. One cup of tea contains between 100 an 200 mg of catechins(Ahmad and Muktar, 1999), which is suggested to account for at least 90% of the observed beneficial effects of green tea.(Williams et al., 2000)

Watercress

Watercress (Nasturtium officinale), like other crucifers, contains high levels of glucosinolates. Glucosinolates are precursors to several bioactive isothiocyanates, including phenylethyl isothiocyanate (PEITC), and watercress is a particularly rich source of PEITC. In humans, research has shown that glucosinolates can be effectively converted to PEITC by gut flora after consumption of watercress.(Getahun and Chung, 1999; Krul et al., 2002) PEITC from watercress has been shown to inhibit chemically-induced lung and colon carcinogenesis in rats, and promote excretion of carcinogens in humans.(Hecht, 1999; Chung et al., 2000) The proposed mechanisms for these activities include inhibition of select Phase I activities with concomitant induction of Phase II glucuronosyl transferases and glutathione S-transferases.(Getahun and Chung, 1999; Rose et al., 2000; Leclercq, 1998) This bifunctional activity of watercress is one of the proposed reasons that epidemiological data shows crucifers to be chemoprotective.

Balance and Healthy Detoxification: Providing a full spectrum of Phase II cofactors

The depletion or insufficiency of any cofactor needed in the detoxification process is a significant factor in susceptibility to toxicity. Phase I prepares a toxin for conjugation by the Phase II system, and Phase II conjugates a water-soluble group to the toxin, rendering it non-toxic and promoting its excretion. These two activities work in concert, and must be balanced. In particular, Phase II activities must be able to keep-up with Phase I or an imbalance in production of reactive intermediates occurs. When Phase I generates a reactive intermediate that is not immediately conjugated and removed, this reactive intermediate can act as a reactive oxygen species and bind DNA, proteins, and RNA, causing irreversible damage to a cell.

There are many Phase II activities, and support for all of these activities is essential to achieve healthy, balanced, complete detoxification. Therefore, high Phase II activity, and a full spectrum of cofactors for Phase II activities are required. Provision of the amino acid conjugation cofactors, which include glycine, glutamine (from protein), and taurine, is important. Providing sulfation cofactors is particularly important since serum sulfate can be easily decreased after toxic exposure. For instance, subchronic acetaminophen (650 mg) doses over four consecutive days resulted in a decrease in serum sulfate levels in healthy subjects.(Hoffman et al., 1990) Sulfate cofactors also support production of glutahione. Methylation support is particularly important for optimal, full spectrum

Phase II activity since methylation is a key player in excretion of steroids and steroid-like toxins.

Sulfation Support with N-Acetylcysteine and Sodium Sulfate

Oral N-acetylcysteine has been shown to increase the level of glutathione produced in the body, which is not only the cofactor for glutathione conjugation, but is also a major route for detoxification of heavy metals by the ability of metals bind to the sulfur in glutathione.(Olmstead, 2000) Due to its support for glutathione production, cysteine becomes depleted in the presence of a toxic load of metals, and it is a principle support factor in combating metal toxicity.(Quig, 1998) Provision of sulfate cofactors with cysteine (provided as N-acetylcysteine) at 200 mg to 500 mg per day is suggested to support sulfation cofactor status and glutathione production.

Methylation and the Labile Methyls in Detoxification

The methyl donors, choline, methionine, and folate, are called "labile methyls" because they are used during metabolism and, therefore, required to be replenished. Interestingly, dietary deficiency of these "labile methyls" is the only nutrient deficiency known to be carcinogenic in itself.(Rogers, 1995) Some Phase I enzymes have also been shown to be adversely induced in animals deficient of dietary labile methyls.(Zhang et al., 1997) The role of these dietary labile methyls in healthy promotion is due, in part, to their important role in supporting balanced biotransformation by providing cofactors for Phase II conjugation reactions. Vitamin B12 and folate provide support for the homocysteine cycle, which allows for remethylation of SAM. The biologically-active, natural form of folate is 5-methyltetrahydrofolate.(Scott, 2001)

Provision of choline is particularly important. Because choline can be synthesized endogenously from methionine, it has been assumed dietary sources are not required; however, much experimental data has challenged this assumption and shown that dietary sources of choline are essential. For example, choline deficiency has been shown to result in fatty liver and other liver diseases.(Buchman et al., 2001; Zeisel, 2000) Recently, the Food and Nutrition Board of the National Academy of Sciences has designated choline as an essential nutrient.(Miller, 2002)

Healthy liver function and antioxidant supports to protect from oxidative stress are essential in detoxification

As can be seen by the above discussion, generation of ATP is vital for adequate detoxification. In particular, Phase II requires large amounts of ATP. Generation of

adequate ATP requires healthy, nutrient-supported mitochondria. Unfortunately, many toxicants can inhibit mitochondrial function, which can lead to a decreased capacity to biotransform other toxins.(Umeda et al., 2000) Production of reactive oxygen species is also a consequence of energy production, and excess presence of these damaging molecules, called oxidative stress, is associated with toxicity. For example, oxidant damage via nitric oxide or peroxynitrite has been implicated in MCS, CFS, and Parkinson's disease, among others.(Pall and Slatterle, 2001; Marshall et al., 1999) Nutrients that support mitochondrial function include the essential cofactors for energy production: thiamin, riboflavin, niacin, pantothenic acid, and magnesium. In addition, nutrients that help protect from oxidative stress, such as vitamins C and E, zinc, selenium, and copper, are also beneficial. (Aw and Jones, 1989; Aruoma, 1994)

In addition to focused antioxidant support, protection of the liver from oxidative stress damage and provision phytonutrients and botanicals that support healthy liver function is particularly important in detoxification.

Silymarin

Several recent reviews have discussed the use of silymarin as a hepatoprotectant.(Saller et al., 2001; Wellington and Jarvis, 2001) For example, silymarin at around 400 mg per day has been shown to improve liver function in patients with various etiologies of liver disease, including those exposed to toxic levels of industrial phenolics, such as toluene. Silymarin has also been shown to increase serum glutathione and glutathione peroxidase in patients with liver disease, and induce glutathione transferase activity in animals. Silymarin glycosides also support strong antioxidant activities,(Kosina et al., 2002) therefore, silymarin may act as a bifunctional modulator.

Artichoke

Traditional medicine has long used artichoke extracts as a hepatoprotectant, and several bioactives in artichoke extract have been identified, including chlorogenic acid, cynarin, caffeic acid, and luteolin.(Perez-Garcia et al, 2000; Llorach et al., 2002) Consumption of encapsulated artichoke extract has been shown to result in absorption of these bioactives in humans, resulting in the production of beneficial metabolites such as ferulic acid.(Rechner et al 2001) Ferulic acid, chlorogenic acid and cynarin provide strong antioxidant protection, which may account for some of their health-promoting activities. Moreover, in cultured liver cells, artichoke extract not only provided antioxidant protection from a toxic chemically-induced insult, but also showed diminished loss of cellular glutathione reserves.(Gebhardt, 1997)

Digestion and Excretion Promote Healthy Detoxification

Healthy digestion can have a profound effect on detoxification. Food intake alters gastric emptying and intestinal transit, pH, and bile secretion.(Singh, 1999) Toxins that are conjugated in the intestinal tract and during first pass metabolism in the liver are primarily excreted via bile, which requires healthy fecal production. In addition, adequate intake of water is essential to maintain healthy kidney function and promote urinary excretion of toxins already in circulation.

A source of fiber and healthy excretion are important to maintain removal of biotransformed toxins. Fiber can benefit a detoxification program in many ways. Fiber supports intestinal mucosal cell barriers and colonic health, which decrease toxic burden on the body and provide a first line of defense to the system. Fiber promotes removal of the conjugated toxins that are excreted via bile, and may decrease the absorption of some toxins. Most notably, fibers in rice bran have been shown to preferentially bind mutagens over wheat, corn, barley, or oat fibers, thereby removing the toxins before they can even interact with the body and cause damage at any level.(Harris et al., 1998)

Summary

A recent New York Academy of Sciences report indicates that individual response to toxin exposure is varied and is a primary factor in susceptibility to toxin-related conditions.(Bell et al., 2001) Several recent reviews have also summarized the current literature on susceptibilities to cancer, and by far the most common enzyme system to be implicated in protection from carcinogens in the environment is the detoxification, or biotransformation, system.(Clapper, 2000; Ingelman-Sundberg, 2001) Each of us has our own unique combination of these varied, multiple enzymes that compose detoxification; however, all of these enzymes require targeted, phytonutrient support to function optimally. Given the magnitude of exposure we encounter everyday, support for detoxification is one way to promote health and manage myriad conditions with toxicity as a central factor.

References

Abbas AB, Clifford MN, Walker R, Ioannides C. Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation by green tea. Carcinogenesis. 1994;15(11):2575-79.

Agarwal R, Mukhtar H. Cancer chemoprevention by polyphenols in green tea and artichoke. Adv Exp Med Biol. 1996;401:35-50.

Ahmad N, Muktar H. Green tea polyphenols and cancer: biological mechanisms and practical implications. Nutr Rev. 1999;57(3):78-83.

Ahmed S, Rahman A, Saleem M, Athar M, Sultana S. Ellagic acid ameliorates nickel induced biochemical alterations: diminution of oxidative stress. Human Exp Toxicol. 1999;18:691-98.

Ahn D, Putt D, Kresty L, Stoner GD, Fromm D, Hollenberg PF. The effects of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes P450 and phase II enzymes. Carcinogenesis. 1996;17(4):821-28.

Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxic. 1994;32:671-83.

Aw TY, Jones DP. Nutrient supply and mitochondrial function. Annu Rev Nutr. 1989;9:229-51.

Barch DH, Rundhaugen LM, Stoner GD, Pillay NS, Rosche WA. Structure-function relationships of the dietary anticarcinogen ellagic acid. Carcinogenesis. 1996;17(2):265-69.

Bell IR, Baldwin JC, Schwartz GE. Sensitization studies in chemically intolerant individuals: implications for individual difference research. Ann N Y Acad Sci. 2001;933:38-47.

Buchman AL, Ament ME, Sohel M, et al. Choline deficiency causes reversible hepatic abnormalities in patients receiving parenteral nutrition: Proof of a human choline requirement: A placebo-controlled trial. J Parenteral Enteral Nutr. 2001;25:260-68.

Buckley JD, Meadows AT, Kadin ME, Le Beau MM, Siegel S, Robison LL. Pesticide exposures in children with non-Hodgkin lymphoma. Cancer. 2000;89(11):2315-21.

CDC. National Center for Health Statistics. 2000.; NCI. SEER Cancer Statistics Review, 1973-1997.

Checkoway H, Powers K, Smith-Weller T, Franklin GM, Longstreth WT Jr, Swanson PD. Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake. Am J Epidemiol. 2002;155(8):732-38.

Chung FL, Conaway CC, Rao CV, Reddy BS. Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. Carcinogenesis. 2000;21(12):2287-91.

Clapper ML. Genetic polymorphism and cancer risk. Curr Oncol Rep. 2000;2(3):251-6.

Daniels JL, Olshan AF, Savitz DA. Pesticides and childhood cancers. Environ Health Perspect. 1997;105(10):1068-77.

Dashwood RH, Xu M, Hernaez JF, Hasaniya N, Youn K, Razzuk A. Cancer chemopreventive mechanisms of tea against heterocyclic amine mutagens from cooked meat. Proc Soc Exp Biol Med. 1999;220(4):239-43.

DeGaetano A, Castagneto M, Mingrone G, et al. Kinetics of the medium-chain triglycerides and free fatty acids in healthy volunteers and surgically stressed patients. J Parenteral Enteral Nutr. 1994;18:134-40.

Fry JR, Sinclair D, Piper CH, Townsend SL, Thomas NW. Depression of glutathione content, elevation of CYP2E1-dependent activation, and the principal determinant of the fasting-mediated enhancement of 1,3-dichloro-2-propanol hepatotoxicity in the rat. Food Chem Toxicol. 1999;37(4):351-55.

Gebhardt R. Antioxidant and protective properties of extracts from leaves of the artichoke (Cynara scolymus L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. Toxicol Appl Pharmacol. 1997;144:279-86.

Getahun SM, Chung F-L. Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. Cancer Epidemiol Biomarkers Prev. 1999;8:447-51.

Goto K, Kanaya S, Ishigami T, Hara Y. The effects of tea catechins on fecal conditions of elderly residents in a long-term care facility. J Nutr Sci Vitaminol. 1999;45:135-41

Harris PJ, Sasidharan VK, Roberton AM, Triggs CM, Blakeney AB, Ferguson LR. Adsorption of a hydrophobic mutagen to cereal brans and cereal bran dietary fibres. Mutation Res. 1998;412:323-31.

Hecht SS. Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. J Nutr. 1999;129:768S-74S.

Hoffman DA, Wallace SM, Verbeeck RK. Circadian rhythm of serum sulfate levels in man and acetaminophen pharmacokinetics. Eur J Clin Pharmacol. 1990;39(2):143-48.

Ingelman-Sundberg M. Genetic variability in susceptibility and response to toxicants. Toxicol Lett. 2001;120(1-3):259-68.

Kall MA, Clausen J. Dietary effect on mixed function P450 a!2 activity assayed by estimation of caffeine metabolism in man. Human Exp Toxicol. 1995;14:801-7.

Khanduja KL, Gandhi RK, Pathania V, Syal N. Prevention of N-nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in mice. Food Chem Toxicol. 1999;37(4):313-18.

Kono H, Enomoto N, Connor HD, et al. Medium-chain triglycerides inhibit free radical formation and TNF-alpha production in rats given enteral ethanol. Am J Physiol Gastrointest Liver Physiol. 2000;278:G467-G76.

Kosina P, Kren V, Gebhardt R, Grambal F, Ulrichova J, Walterova D. Antioxidant properties of silybin glycosides. Phytotherapy Res. 2002;16:S33-S39.

Krul C, Humblot C, Phillippe C, et al. Metabolism of sinigrin (2-propenyl glucosinolate) by the human coloni microflora in a dynamic in vitro large-intestine model. Carcinogenesis. 2002;23:1009-16.

Lall SB, Singh B, Gulati K, Seth SD. Role of nutrition in toxic injury. Indian J Exp Biol. 1999;37(2):109-16.

Leclercq I, Desager JP, Horsmans Y. Inhibition of chlorzoxazone metabolism, a clinical probe for CYP2E1, by a single ingestion of watercress. Clin Pharmacol Ther. 1998;64(2):144-49.

Levin B. Environmental Nutrition. Vashon Is, WA:HingePin Press;1999:179-84.

Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer. N Eng J Med. 2000;343:78-85.

Liska DJ. The detoxification enzyme systems. Altern Med Rev. 1998;3(3):187-98.

Llorach R, Espin JC, Thomas-Barberan FA, Ferreres F. Artichoke (Cynara scolymus L.) byproducts as a potential source of health-romoting antioxidant phenolics. J Agric Food Chem. 2002;50:3458-64.

Marshall K-A, Reist M, Jenner P, Halliwell B. The neuronal toxicity of sulfite plus peroxynitrite is enhanced by glutathione depletion: implications for Parkinson's disease. Free Rad Biol Med. 1999;27:515-20.

McDanell RE, Henderson LA, Russell K, McLean AEM. The effect of Brassica vegetable consumption on caffeine metabolism in humans. Human Exp Toxicol. 1992;11:167-172.

McKay DL, Blumberg JB. The role of tea in human health: an update. J Am Coll Nutr. 2002;21(1):1-13.

Meinert R, Schuz J, Kaletsch U, Kaatsch P, Michaelis J. Leukemia and non-Hodgkin's lymphoma in childhood and exposure to pesticides: results of a register-based case-control study in Germany. Am J Epidemiol. 2000;151(7):639-46, 647-50.

Miller DL. Health benefits of lecithin and choline. Cereal Foods World. 2002;47:178-84.

Muir T, Zegarac M. Societal costs of exposure to toxic substances: economic and health costs of four case studies that are candidates for environmental causation. Environ Health Perspect. 2001;109Suppl 6:885-903.

Olmstead MJ. Heavy metal sources, effects, and detoxification. Altern Ther Complement Med. 2000;Dec;347-354.

Pall ML, Satterle JD. Elevated nitric oxide/peroxynitrite mechanism for the common etiology of multiple chemical sensitivity, chronic fatigue syndrome, and posttraumatic stress disorder. Ann N Y Acad Sci. 2001;933:323-29.

Perez-Garcia F, Adzet T, Canigueral S. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. Free Rad Res. 2000;33:661-65.

Quig D. Cysteine metabolism and metal toxicity. Altern Med Rev. 1998;3(4):262-70.

Racciatti D, Vecchiet J, Ceccomancini A, Ricci F, Pizzigallo E. Chronic fatigue syndrome following a toxic exposure. Sci Total Environ. 2001;270(1-3):27-31.

Rechner AR, Pannala AS, Rice-Evans CA. Caffeic acid derivatives in artichoke extract are metabolised to phenolic acids in vivo. Free Rad Res. 2001;35:195-202.

Rogers AE. Methyl donors in the diet and responses to chemical carcinogens. Am J Clin Nutr. 1995;61(Suppl):659S-65S.

Rose P, Faulkner K, Williamson G, Mithen R. 7-Methylsulfinylheptyl and 8-methylsulfinyloctyl isothiocyanates from watercress are potent inducers of phase II enzymes. Carcinogenesis. 2000;21(11):1983-88.73.

Ross GW, Abbott RD, Petrovitch H, et al. Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA. 2000;283:2674-79.

Rothman N, Cantor KP, Blair A, et al. A nested case-control study of non-Hodgkin lymphoma and serum organochlorine residues. Lancet. 1997;350:240-44.

Rudel RA, Brody JG, Spengler JD, et al. Identification of selected hormonally active agents and animal mammary carcinogens in commercial and residential air and dust samples. J Air Waste Mange Assoc. 2001;51(4):499-513.

Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs. 2001;61(14):2035-63.

Scott J. Methyltetrahydrofolate: the superior alternative to folic acid. In:Kramer K, Hoppel P-P, eds. Nutraceuticals in Health and Disease Prevention. New York:Marvel Dekker, 2001;6:75-90.

Sherer TB, Betarbet R, Greenamyre JT. Environment, mitochondria, and Parkinson's disease. Neuroscientist. 2002;8(3):192-7.

Silkworth JB, Brown JF Jr. Evaluating the impact of exposure to environmental contaminants on human health. Clin Chem. 1996;42:8(B):1345-49.

Singh BN. Effects of food on clinical pharmacokinetics. Clin Pharmacokinet. 1999;37(3):213-55.

Singh K, Khanna AK, Chander R. Hepatoprotective activity of ellagic acid against carbon tetrachloride induced hepatotoxicity in rats. Indian J Exp Biol. 1999;37(10):1025-26.

Steele VE, Kelloff GJ, Balentine D, et al. Comparative chemopreventive mechanisms of green tea, black tea, and selected polyphenol extracts measured by in vitro bioassays. Carcinogenesis. 2000;21(1):63-7.

Szende B, Timar F, Hargitai B. Olive oil decreases liver damage in rats caused by carbon tetrachloride (CCl4). Exp Toxicol Pathol. 1994;46:355-59.

Umeda S, Muta T, Ohsato T, Takamatsu C, Hamasaki N, Kang D. The D-loop structure of human mtDNA is destabilized directly by 1-methyl-4-phenylpyridinium ion (MPP+), a parkinsonism-causing toxin. Eur J Biochem. 2000;267:200-06.

Vanden Heuvel JP, Clark GC, Kohn MC, et al. Dioxin-responsive genes: examination of dose-response relationships using quantitative reverse transcriptase-polymerase chain reaction. Cancer Res. 1994;54:62-8.

Webster LR, McKenzie GH, Moriarty HT. Organophosphate-based pesticides and genetic damage implicated in bladder cancer. Cancer Genet Cytogenet. 2002;133(2):112-17.

Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. BioDrugs. 2001;15(7):465-89.

Williams SN, Shih H, Guenette DK, et al. Comparative studies on the effects of green tea extracts and individual tea catechins on human CYP1A gene expression. Chem Biol Interact. 2000;128(3):211-29.

Xu M, Bailey AC, Hernaez JF, Taoka CR, Schut HAJ, Dashwood RH. Protection by green tea, black tea, and indole-3-carbinol against 2-amino-3-methylimidazo[4,5-f]quinoline-induced DNA adducts and colonic aberrant crypts in the F344 rat. Carcinogenesis. 1996;17(7):1429-34.

Zeisel SH. Choline: an essential nutrient for humans. Nutrition. 2000;16:669-71.

Zhang J, Henning SM, Heber D, et al. NADPH-cytchrome P-450 reductase, cytochrome P-450 2C11 and P450 A1, and the aryl hydrocarbon receptor in livers of rats fed methyl-folate-deficient diets. Nutrition. 1997;28:160-64.