COURSE NOTES: Understanding genetics for improving health outcomes

Course Code: CEUGH

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Course Description

The course is intended to provide the tools that holistic nutritionists can use to deliver personalized healthcare to their clients, catered to the genetic makeup of the client. Genetics plays a very robust role in nutrition, detoxification, weightloss and overall health and wellness. Furthermore, genetics can be used not only to improve the health of the clients but also to develop DNA based nutritional plans that can potentially prevent development of chronic diseases. Course participants will gain knowledge on how genetic information can be used to deliver nutritional plans, weightloss strategies, detoxification plans, hormonal balance plans along with nutritional plans to prevent development of chronic disease such as diabetes and cardiovascular diseases.

IHN has partnered with Anantlife Canada Inc., a leader in clinical grade genetic testing for healthcare providers all over the world, to offer a Certified Genetic Testing Provider Certificate upon successful completion of the course. Successful completion of the course implies that the candidates have received the education and training to not only understand genetic concepts pertaining to diet, nutrition, detoxification, fitness, hormonal health and metabolic disorders but have also been trained on interpretation of the genetic testing reports along with development of a DNA based health plan for better health outcomes.

SESSION 4:

DETOXIFICATION GENETICS: UNDERSTAND THE GENETICS OF DETOXIFICATION AND THE MODULATION OF DETOXIFICATION PATHWAYS BY NUTRITIONAL/DIETARY INTERVENTION. HOW CAN UNDERSTANDING DETOXIFICATION PATHWAYS BE APPLIED TO IMPROVE HEALTH?

Detoxification is carried out in the liver, largely via a multi-enzyme complex called Cytochrome p450 complex encoded by several genes. All chemicals, hormones, pollutants, toxins are eliminated via this system and as such genetics plays an essential role in determining detoxification potential. However, detoxification pathways are amenable to dietary intervention, whereby if an enzymatic activity is impaired (which can be identified by analyzing DNA), dietary intervention can boost activity of the enzyme by impacting epigenetics. The readings herein are to provide an extensive understanding of detoxification pathways along with how dietary intervention can be used to modulate detoxification pathways.

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Review Article

Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application

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Research into human biotransformation and elimination systems continues to evolve. Various clinical and *in vivo* studies have been undertaken to evaluate the effects of foods and food-derived components on the activity of detoxification pathways, including phase I cytochrome P450 enzymes, phase II conjugation enzymes, Nrf2 signaling, and metallothionein. This review summarizes the research in this area to date, highlighting the potential for foods and nutrients to support and/or modulate detoxification functions. Clinical applications to alter detoxification pathway activity and improve patient outcomes are considered, drawing on the growing understanding of the relationship between detoxification functions and different disease states, genetic polymorphisms, and drug-nutrient interactions. Some caution is recommended, however, due to the limitations of current research as well as indications that many nutrients exert biphasic, dose-dependent effects and that genetic polymorphisms may alter outcomes. A whole-foods approach may, therefore, be prudent.

1. Introduction

Food-based nutrients have been and continue to be investigated for their role in the modulation of metabolic pathways involved in detoxification processes. Several publications to date have leveraged cell, animal, and clinical studies to demonstrate that food-derived components and nutrients can modulate processes of conversion and eventual excretion of toxins from the body [1]. In general, the nature of these findings indicates that specific foods may upregulate or favorably balance metabolic pathways to assist with toxin biotransformation and subsequent elimination [2, 3]. Various whole foods such as cruciferous vegetables [2, 4, 5], berries [6], soy [7], garlic [8, 9], and even spices like turmeric [10, 11] have been suggested to be beneficial and commonly prescribed as part of naturopathic-oriented and functional medicine-based therapies [12, 13].

While these foods are important to note, the science in this active area of inquiry continues to evolve to reveal new findings about food-based nutrients and their effect on health. Thus, the purpose of this review article is to summarize the science to date on the influence of whole foods, with a special focus directed towards phytonutrients and other food-based components, on influencing specific metabolic detoxification pathways, including phase I cytochrome enzymes, phase II conjugation enzymes, antioxidant support systems, and metallothionein upregulation for heavy metal metabolism. Based on this current science, the paper will conclude with clinical recommendations that may be applied in a personalized manner for patients via the discretion of a qualified health professional.

2. The Metabolic Pathways of Detoxification

Discussion of physiological pathways for detoxification has been mainly centered around phase I and phase II enzyme systems. This review will cover phase I cytochrome P450

enzymes as well as phase II enzymes, specifically UDP-glucuronosyl transferases, glutathione S-transferases, amino acid transferases, N-acetyl transferases, and methyltransferases. Note that there are other important classes of phase I enzymes, namely, hydroxylation and reduction, which are not covered in this review. While these important enzymes are pivotal to consider, this review of the effect of food on detoxification will also extend into other pathways, including ways to promote gene expression of antioxidant-related enzymes and of metallothionein, an endogenous protein carrier for heavy metals. Each of these four classes of detoxification-related pathways will be discussed within the context of nutrients.

2.1. Phase I Cytochrome P450 Enzymes. Initially, the "phases" of detoxification were described as functionalization (or phase I), or the addition of oxygen to form a reactive site on the toxic compound, and conjugation (phase II), or the process of adding a water-soluble group to this now reactive site [14, 15]. The "Phase I" cytochrome P450 superfamily of enzymes (CYP450) is generally the first defense employed by the body to biotransform xenobiotics, steroid hormones, and pharmaceuticals. These microsomal membrane-bound, heme-thiolate proteins, located mainly in the liver, but also in enterocytes, kidneys, lung, and even the brain, are responsible for the oxidation, peroxidation, and reduction of several endogenous and exogenous substrates [13, 15, 16]. Specifically, the function of CYP450 enzymes is to add a reactive group such as a hydroxyl, carboxyl, or an amino group through oxidation, reduction, and/or hydrolysis reactions [15]. These initial reactions have the potential to create oxidative damage within cell systems because of the resulting formation of reactive electrophilic species.

It is accepted that any variability in the number of CYP450 enzymes could have benefit(s) and/or consequence(s) for how an individual responds to the effect(s) of (a) toxin(s). Clinical application of the knowledge of these phase I CYP450 enzymes has been primarily addressed within pharmacology to understand the nature of drug interactions, side effects, and interindividual variability in drug metabolism [15]. The ability of an individual to metabolize 90% of currently used drugs will largely depend on the genetic expression of these enzymes [17]. It is established that many of these CYP450 genes are subject to genetic polymorphisms, resulting in an altered expression and function of individual enzymes. Currently, there exist some laboratory tests to identify the presence of these genetic variants. It is conceivable that having knowledge about foods and their individual (phyto)nutrients, especially in the case of dietary supplements and functional foods, could be worthwhile for clinicians to consider for patients who are taking a polypharmacy approach. Furthermore, as nutritional strategies become more personalized, it would seem that this information could be interfaced with a patient's known CYP450 polymorphisms to determine how to best optimize health outcomes.

2.1.1. CYP1 Enzymes. The CYP1A family is involved in metabolizing procarcinogens, hormones, and pharmaceuticals. It is well-known for its role in the carcinogenic bioactivation of polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines/amides, polychlorinated biphenyls (PCBs), and other environmental toxins [18, 19]. Low CYP1A2 activity, for example, has been linked to higher risk of testicular cancer [20]. However, due to their rapid conversion to highly reactive intermediates, excessive activity of CYP1A enzymes without adequate phase II support may enhance the destructive effects of environmental procarcinogens [21]. Indeed, genetic polymorphisms in this cytochrome family have been suggested as useful markers for predisposition to certain cancers [15]. CYP1 enzymes are also involved in the formation of clinically relevant estrogen metabolites: CYP1A1/1A2 and CYP1B1 catalyze the 2-hydroxylation and 4-hydroxylation of estrogens, respectively [22]. The potential role of 4-hydroxyestradiol in estrogen-related carcinogenesis, via the production of free radicals and related cellular damage [22], has prompted investigation into factors that modulate CYP1 enzymes.

Various foods and phytonutrients alter CYP1 activity (Tables 1(a) and 1(b)). Cruciferous vegetables have been shown, in humans, to act as inducers of CYP1A1 and 1A2, and animal studies also suggest an upregulation of CYP1B1 [4, 23–27]. The inductory effect of crucifers on CYP1A2 seems especially well established. Clinical studies also indicate that resveratrol and resveratrol-containing foods are CYP1A1 enhancers [28]. Conversely, berries and their constituent polyphenol, ellagic acid, may reduce CYP1A1 overactivity [6], and apiaceous vegetables and quercetin may attenuate excessive CYP1A2 action [24, 29]. Cruciferous vegetables and berries have been suggested as possible modulators of estrogen metabolites: berries for their reducing effect on CYP1A1 [6] and cruciferous vegetables for their stronger induction of CYP1A versus 1B1 enzymes [25–27, 30]. Chrysoeriol, present in rooibos tea and celery, acts selectively to inhibit CYP1B1 *in vitro* [31] and may be especially relevant to patients with CYP1B1 overactivity. However, further research is needed to confirm this finding.

Many foods appear to act as both inducers and inhibitors of CYP1 enzymes, an effect which may be dose dependent or altered by the isolation of bioactive compounds derived from food. Curcumin at 0.1% of the diet has been shown, in animals, to induce CYP1A1, for example, [35], yet a diet of 1% turmeric was inhibitory [46]. Black tea at 54 mL/d induced both CYP1A1 and 1A2 [33], yet 20 mg/kg of theaflavins was inhibitory to CYP1A1 [45]. Soybean intake at 100 mg/kg upregulated CYP1A1 activity [7], yet at 1 g/kg black soybean extract [44] and 200 mg daidzein twice daily [49], its effect was inhibitory. Further research is needed to confirm different dose effects and impact in humans.

Varied effects may also occur from different members of the same food group. Seemingly contradictory to research showing that cruciferous vegetables activate CYP1 enzymes, kale (another member of the cruciferous family) appears to inhibit CYP1A2 (as well as 2C19, 2D6, and 3A4) in animals [51]. The dose used, at 2 g/kg per day, is 15-fold higher than the typical level of human consumption [51], and more research would be required to determine whether lower intake levels would also have a similar effect. The same authors also tested

Table 1: (a) Human and *in vivo* example nutrient inducers of CYP1 enzymes. (b) Human and *in vivo* example nutrient inhibitors of CYP1 enzymes.

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP1A1	Cruciferous vegetables	Clinical	500 mg/d indole-3-carbinol [23]
	Resveratrol *Grapes, wine, peanuts, soy*, and *itadori tea* [32]	Clinical	1 g/d resveratrol [28]: *note high dose used*
	Green tea	*In vivo*	45 mL/d/rat (avg. 150 g animal weight) green tea [33]
	Black tea	*In vivo*	54 mL/d/rat (avg. 150 g animal weight) black tea [33]
	Curcumin *Turmeric, curry powder* [34]	*In vivo*	1,000 mg/kg/d/rat curcumin [35], or about 150 mg per rat per day
	Soybean	*In vivo*	100 mg/kg soybean extract [7]
	Garlic	*In vivo*	30 to 200 mg/kg garlic oil [36]
	Fish oil	*In vivo*	20.5 g/kg fish oil [36]: *note high dose used*
	Rosemary	*In vivo*	Diet of 0.5% rosemary extract [37]
	Astaxanthin *Algae, yeast, salmon, trout, krill, shrimp*, and *crayfish* [38]	*In vivo*	Diets of 0.001–0.03% astaxanthin for 15 days [39]
CYP1A2	Cruciferous vegetables	Clinical	7–14 g/kg cruciferous vegetables including frozen broccoli and cauliflower, fresh daikon radish sprouts and raw shredded cabbage, and red and green [24] 500 g/d broccoli [4] 250 g/d each of Brussel sprouts and broccoli [25] 500 g/d broccoli [26]
	Green tea	*In vivo*	45 mL/d/rat (avg. 150 g animal weight) green tea [33] Green tea (2.5% w/v) as sole beverage [40]
	Black tea	*In vivo*	54 mL/d/rat (avg. 150 g animal weight) black tea [33]
	Chicory root	*In vivo*	Diet of 10% dried chicory root [41]
	Astaxanthin *Algae, yeast, salmon, trout, krill, shrimp*, and *crayfish* [38]	*In vivo*	Diets of 0.001–0.03% astaxanthin for 15 days [39]
CYP1B1	Curcumin *Turmeric, curry powder* [34]	*In vivo*	Diet of 0.1% curcumin [35]
	Cruciferous vegtables	*In vivo*	25–250 mg/kg indole-3-carbinol [27]

(b)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP1A1	Black raspberry	*In vivo*	Diet of 2.5% black raspberry [6]
	Blueberry	*In vivo*	Diet of 2.5% blueberry [6]
	Ellagic acid *Berries, pomegranate, grapes, walnuts*, and *blackcurrants* [42]	*In vivo*	30 mg/kg/d ellagic acid [43] 400 ppm ellagic acid [6]
	Black soybean	*In vivo*	1 g/kg black soybean seed coat extract [44]: *note high dose used*
	Black tea	*In vivo*	20 mg/kg theaflavins [45]
	Turmeric	*In vivo*	Diet of 1% turmeric [46]

(b) Continued.

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP1A2	Apiaceous vegetables	Clinical	4 g/kg apiaceous vegetables, including frozen carrots and fresh celery, dill, parsley, and parsnips [24]
	Quercetin *Apple, apricot, blueberries, yellow onion, kale, alfalfa sprouts, green beans, broccoli, black tea*, and *chili powder* [47, 48]	Clinical	500 mg/d quercetin [29]
	Daidzein *Soybean* [49]	Clinical	200 mg twice daily dosing of daidzein [49]
	Grapefruit	Clinical	300 mL grapefruit juice [50]
	Kale	*In vivo*	2 g/kg/d kale, as freeze-dried kale drink [51]
	Garlic	*In vivo*	100 mg/kg garlic oil [52]
	Chamomile	*In vivo*	Free access to 2% chamomile tea solution [53]
	Peppermint	*In vivo*	Free access to 2% peppermint tea solution [53]
	Dandelion	*In vivo*	Free access to 2% dandelion tea solution [53]
	Turmeric	*In vivo*	Diet of 1% turmeric [46]

the effects of an equivalent volume of cabbage consumption and found no such inhibitory effect, pointing to the possibility that different cruciferous vegetables may have distinct effects on cytochrome activity.

2.1.2. CYP2A-E Enzymes. The large CYP2 family of enzymes is involved in the metabolism of drugs, xenobiotics, hormones, and other endogenous compounds such as ketones, glycerol, and fatty acids [15, 54]. Some notable polymorphisms occur in the CYP2C and CYP2D subgroups, leading to the classification of patients as "poor metabolizers" of various pharmaceuticals: warfarin and CYP2C9, antiarrhythmia agents, metoprolol and propafenone, and CYP2D6, phenytoin, cyclobarbital, omeprazole, and CYP2C19, for example, [15, 17]. CYP2D polymorphisms may be associated with Parkinson's disease and lung cancer [15]. Clinical evidence exists for the induction of CYP2A6 by quercetin and broccoli [4, 29] (Table 2(a)). In animals, chicory appears to induce CYP2A enzymes [41] and rosemary and garlic may upregulate CYP2B activity [9, 37]. Clinical studies using resveratrol and garden cress indicate CYP2D6 inhibition [28, 55] (Table 2(b)). Ellagic acid, green tea, black tea, and cruciferous vegetables also appear to inhibit various CYP2 enzymes.

CYP2E1 enzymes have also attracted particular interest for their role in various diseases. 2E1 metabolizes nervous system agents such as halothane, isoflurane, chlorzoxazone, and ethanol and bioactivates procarcinogenic nitrosamines and aflatoxin B1 [15, 65]. It produces free radicals regardless of substrate [15], and CYP2E1 polymorphisms have been associated with altered risk for coronary artery disease [66] and gastric cancer [67]. CYP2E1-induced oxidative stress has also been shown to lead to impaired insulin action via the suppression of GLUT4 expression [68]. Attenuation of 2E1 overactivity may therefore be an important consideration in high-risk patients.

Watercress and garlic are CYP2E1 inhibitors in humans [59, 60]. *In vivo* evidence also suggests that N-acetyl cysteine, ellagic acid, green tea, black tea, dandelion, chrysin, and medium chain triglycerides (MCTs) may downregulate CYP2E1 [33, 43, 54, 61, 63, 64]. MCT oil may specifically attenuate the ethanol-induced upregulation of CYP2E1 and production of mitochondrial 4-hydroxynonenal, a marker of oxidative stress [64].

2.1.3. CYP3A Enzymes. The occurrence of the different CYP3A isoforms is tissue-specific [15]. Rooibos tea, garlic, and fish oil appear to induce the activity of CYP3A, 3A1, and 3A2 [8, 36, 69, 70] (Table 3(a)). Possible inhibitory foods include green tea, black tea, and quercetin [33, 56, 71, 72] (Table 3(b)). The most clinically relevant of the enzymes is CYP3A4, which is expressed mainly in the liver and to a lesser extent in the kidney [13]. Caffeine, testosterone, progesterone, and androstenedione are substrates of the CYP3A4 enzyme system, as are various procarcinogens including PAHs and aflatoxin B1 [15]. To date, however, the principal driver for research on CYP3A4 has been due to its role in the metabolism of over 50 percent of all pharmaceuticals [73]. The potential for drug interaction with this single enzyme, coupled with the wide interindividual differences in enzymatic activity, generates some level of risk in administration of high doses and multiple drugs as well as food-drug

Table 2: (a) Human and *in vivo* example nutrient inducers of selected CYP2 enzymes. (b) Human and *in vivo* example nutrient inhibitors of selected CYP2 enzymes.

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP2A	Chicory root	*In vivo*	Diet of 10% dried chicory root [41]
CYP2A6	Quercetin *Apple, apricot, blueberries, yellow onion, kale, alfalfa sprouts, green beans, broccoli, black tea*, and *chili powder* [47, 48]	Clinical	500 mg/d quercetin [29]
	Broccoli	Clinical	500 g/d broccoli [4]
CYP2B1	Rosemary	*In vivo*	Diet of 0.5% rosemary extract [37]
	Garlic	*In vivo*	0.5 and 2.0 mmol/kg diallyl sulfide, or about 75 and 300 mg, respectively [9]
CYP2B2	Rosemary	*In vivo*	Diet of 0.5% rosemary extract [37]
CYP2E1	Fish oil	*In vivo*	20.5 g/kg fish oil [36]: *note high dose used*
	Chicory root	*In vivo*	Diet of 10% dried chicory root [41]

(b)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP2B	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	10 and 30 mg/kg/d ellagic acid [43]
	Green tea	*In vivo*	100 mg/kg/d green tea extract [56]
	Cruciferous vegetables	*In vivo*	3 and 12 mg/kg/d sulforaphane [57]
CYP2B1	Turmeric	*In vivo*	Diet of 1% turmeric [46]
CYP2C	Green tea	*In vivo*	45 mL/d/rat (avg. 150 g animal weight) green tea [33]
	Black tea	*In vivo*	54 mL/d/rat (avg. 150 g animal weight) black tea [33]
	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	30 mg/kg/d ellagic acid [43]
CYP2C6	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	30 mg/kg/d ellagic acid [43]
CYP2C9	Resveratrol *Grapes, wine, peanuts, soy,* and *itadori tea* [32]	Clinical	1 g/d resveratrol [28]: *note high dose used*
	Myricetin *Onions, berries, grapes,* and *red wine* [58]	*In vivo*	2 and 8 mg/kg myricetin [58]
CYP2C19	Kale	*In vivo*	2 g/kg/d kale, as freeze-dried kale drink [51]
CYP2D6	Resveratrol *Grapes, wine, peanuts, soy,* and *itadori tea* [32]	*Clinical*	1 g/d resveratrol [28]: *note high dose used*
	Garden cress	Clinical	7.5 g twice daily intake of garden cress seed powder [55]
	Kale	*In vivo*	2 g/kg/d kale, as freeze-dried kale drink [51]

(b) Continued.

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
	Watercress	Clinical	50 g watercress homogenate [59]
	Garlic	Clinical and *in vivo*	0.2 mg/kg diallyl sulfide, equivalent to high human garlic consumption [60] 100 mg/kg garlic oil [52] 200 mg/kg diallyl sulfide [8] 30 to 200 mg/kg garlic oil [36] Diet of 2% and 5% garlic powder [61]
	N-acetyl cysteine *Allium vegetables* [54]	*In vivo*	25 mg/kg and 50 mg/kg N-acetyl cysteine [54]
CYP2E1	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	10 and 30 mg/kg/d ellagic acid [43]
	Green tea	*In vivo*	45 mL/d/rat (avg. 150 g animal weight) green tea [33]
	Black tea	*In vivo*	54 mL/d/rat (avg. 150 g animal weight) black tea [33]
	Dandelion	*In vivo*	0.5 and 2 g/kg dandelion leaf water extract [62]
	Chrysin *Honey, honeycomb* [63]	*In vivo*	20 and 40 mg/kg/d chrysin [63]
	Medium-chain triglycerides (MCTs) *Coconut and coconut oil*	*In vivo*	32% calories as MCTs [64]

and herb-drug interactions. Grapefruit juice is perhaps the most well-known food inhibitor of this enzyme [74], though resveratrol and garden cress, a member of the cruciferous vegetable family, appear to have similar effects in humans, albeit at intakes above what would be expected without high-dose supplementation [28, 55]. Curcumin may upregulate 3A4 activity [11].

Once again, there are indications that a biphasic effect may be seen from dietary bioactive compounds; Davenport and Wargovich (2005) found that shorter-term or lower dosing with garlic organosulfur compounds produced potentially anticarcinogenic effects but that longer-term higher doses (200 mg/kg) of allyl sulfides led to minor hepatic toxicity [8]. One garlic clove contains only 2,500–4,500 μg of the allyl sulfide precursor, allicin [76], so the higher dose is much more than would be consumed in a typical human diet. In another example, two components of cruciferous vegetables, sulforaphanes and indole-3-carbinol, inhibited and increased activity, respectively [57, 75], highlighting the potential for human studies using whole foods to clarify the outcome of consumption.

2.1.4. CYP4 Enzymes. Less is known about this family of enzymes, since it is thought to play a smaller role in drug metabolism. It is, however, understood to be a primarily extrahepatic family of cytochromes, inducible by clofibrate and ciprofibrate (hypolipidemic drugs), NSAIDs, prostaglandins, and toxicants such as phthalate esters [15, 77]. The CYP4B1 isoform is involved in the metabolism of MCTs (medium chain triglycerides), as well as the bioactivation of pneumotoxic and carcinogenic compounds [78].

Polymorphisms and overexpression of this subgroup may be associated with bladder cancer [15] and colitis [79]. A report by Ye et al. (2009) which examined the link between colitis and CYP4B1 activity found that the promotion of CYP4B1 activity by caffeic acid (found in caffeine-containing foods) (Table 4) correlated with reduced inflammation and disease activity [79]. Green tea may act to induce CYP4A1, as suggested by animal studies [40]. More research is needed to clearly identify food influences on this enzyme family.

2.2. Phase II Conjugation Enzymes. After a xenobiotic has gone through the process of becoming hydrophilic through reactions overseen by CYP450 enzymes, its reactive site can be conjugated with an endogenous hydrophilic substance. This reaction is often referred to as "phase II detoxification." Conjugation involves the transfer of a number of hydrophilic compounds (via their corresponding enzymes), including glucuronic acid (glucuronyl transferases), sulfate (sulfotransferases), glutathione (glutathione transferases), amino acids (amino acid transferases), an acetyl group (N-acetyl transferases), and a methyl group (N- and O-methyltransferases) [81]. The result of the collective activity of these enzymes is an increase in the hydrophilicity of the metabolite, theoretically leading to enhanced excretion in the bile and/or urine [81]. Similar to the CYP450 enzymes, genetic polymorphisms can have profound influence on the function of these conjugating

Table 3: (a) Human and *in vivo* example nutrient inducers of selected CYP3 enzymes. (b) Human and *in vivo* example nutrient inhibitors of selected CYP3 enzymes.

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP3A	Rooibos tea	*In vivo*	Rooibos tea, 4 g/L simmered for 5 minutes, as sole beverage [69]
CYP3A1	Garlic	*In vivo*	30 to 200 mg/kg garlic oil [36] 80 and 200 mg/kg garlic oil 3 times weekly [70]
	Fish oil	*In vivo*	20.5 g/kg fish oil [36]: *note high dose used*
CYP3A2	Garlic	*In vivo*	200 mg/kg diallyl sulfide [8]
	Cruciferous vegetables	*In vivo*	50 mg/kg/d indole-3-carbinol [75]
CYP3A4	Curcumin *Turmeric, curry powder* [34]	*In vivo*	50 and 100 mg/kg curcumin [11]

(b)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP3A	Green tea	*In vivo*	45 mL/d/rat (avg. 150 g animal weight) green tea [33] 400 mg/kg green tea extract [71] 100 mg/kg/d green tea extract [56]
	Black tea	*In vivo*	54 mL/d/rat (avg. 150 g animal weight) black tea [33]
	Quercetin *Apple, apricot, blueberries, yellow onion, kale, alfalfa sprouts, green beans, broccoli, black tea,* and *chili powder* [47, 48]	*In vivo*	10 and 20 mg/kg [72]
CYP3A2	Cruciferous vegetables	*In vivo*	12 mg/kg/d sulforaphane [57]
CYP3A4	Grapefruit	Clinical	200 mL grapefruit juice 3 times daily [74]
	Resveratrol *Grapes, wine, peanuts, soy,* and *itadori tea* [32]	Clinical	1 g/d resveratrol [28]: *note high dose used*
	Garden cress	Clinical	7.5 g twice daily dose of garden cress seed powder [55]
	Soybean	*In vivo*	100 mg/kg soybean extract [7]
	Kale	*In vivo*	2 g/kg/d kale, as freeze-dried kale drink [51]
	Myricetin *Onions, berries, grapes,* and *red wine* [58]	*In vivo*	0.4, 2, and 8 mg/kg myricetin [58]

Table 4: Human and *in vivo* example nutrient inducers of selected CYP4 enzymes.

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
CYP4A1	Green tea	*In vivo*	Green tea (2.5% w/v) as sole beverage [40]
CYP4B1	Caffeic acid *Coffee* [80]	*In vivo*	179 mg/kg caffeic acid [79]

enzymes [82], with potential implication in the development of several forms of cancer [83].

It is conceivable that modulation of phase II enzymes by food-based bioactive compounds may be advantageous in patients who have altered enzyme activity due to genetic polymorphisms or who have a high toxic burden due to chronic exposure to environmental pollutants, overactive phase I activity, or hormonal imbalance. For example, James et al. (2008) suggest that upregulation of glucuronidation and sulfonation by certain bioactive compounds may be

a useful consideration for the elimination of environmental PCBs [19].

2.2.1. UDP-Glucuronosyltransferases. This class of enzymes, comprising multiple proteins and even subfamilies, plays an essential role in enhancing the elimination of biotransformed toxins in urine and feces, as well as metabolizing steroid hormones and bilirubin [84, 85]. Their function is to catalyze the covalent linkage of glucuronic acid from UDP-glucuronic acid to an accepting functional group on the molecule, a process referred to as glucuronidation [86]. Glucuronidation occurs primarily in the liver but can occur in other tissues, such as the small intestine [86, 87]. Bilirubin, specifically, is principally conjugated by UGT1A1 in hepatocytes [88] and then excreted with bile into the intestinal tract. It has been estimated that 40–70% of all medications are subject to glucuronidation reactions in humans, thereby suggesting the significance of this conjugation enzyme family [88]. Since UDP-glucuronosyltransferases (UGTs) also metabolize phytochemicals, alterations in their effects may be seen with genetically downregulated enzyme activity; flavonoids are conjugated with glucuronide and sulfate; therefore, UGT or sulfotransferase (SULT) polymorphisms may produce variability in phytochemical clearance and efficacy [89].

Clinical and observational studies point to cruciferous vegetables, resveratrol, and citrus as foods and bioactive compounds that induce UGT enzymes [25, 28, 90–92] (Table 5(a)). Animal studies also suggest the potential for other foods and nutrients, including dandelion, rooibos tea, honeybush tea, rosemary, soy, ellagic acid, ferulic acid, curcumin, and astaxanthin, to enhance UGT activity [37, 39, 53, 93–95]. Interestingly, the effect of resveratrol was seen only in individuals with low baseline enzyme levels/activity, suggesting that some phytochemicals may modulate, rather than outright induce, enzymatic activity [28]. In addition, many studies note that effects are variable depending on gender and genotype [85, 90, 92]; for example, women with the UGT1A1 ∗28 polymorphism (7/7) were responsive to citrus intervention, whereas those with other genetic variants were not [92].

Meaningful interpretations of these studies may still be elusive, however: in one combined dietary trial, the consumption of 10 servings per day of a combination of cruciferous vegetables, soy foods, and citrus fruits did not have a significant effect on UGT enzyme activity compared with a diet devoid of fruits and vegetables [85]. The authors hypothesize that these results may be due to their choice of specific foods within those groups or due to Nrf2 activation (discussed in subsequent sections) when fruits and vegetables were avoided.

The effects of UGT activity may also be enhanced by D-glucaric acid by theoretical inhibition of beta-glucuronidase enzymes [100]. Beta-glucuronidase enzymes act to reverse UGT conjugation reactions. D-glucaric acid is found in many fruits, vegetables and legumes (Table 5(b)). When tested in humans, however, a diet supplemented with cruciferous vegetables (2/3 cup broccoli, 1/2 cup cabbage, and 1/2 cup radish sprouts), citrus fruits (1 cup grapefruit juice, 1/2 cup orange juice, 1 cup orange/grapefruit segments, and 1 orange peel), and soy foods was found to have no effect on beta-glucuronidase activity [101] (amounts standardized for 55 kg body weight), indicating that the clinical effects of D-glucaric acid consumption still need further clarification.

In vivo research suggests that polyphenol extracts of certain berries, specifically strawberries and blackcurrant, may inhibit beta-glucuronidase activity in the intestinal lumen; Kosmala et al. (2014) observed this effect using both strawberry pomace water extract and water-alcohol extract containing 5.1% and 17.1% ellagic acid, and 0.2% and 10.9% proanthocyanidins, respectively [100]. Jurgoński et al. (2014) found a similar inhibitory effect using a diet of 1.5% blackcurrant extract (total polyphenolic content 66.8 g/100 g extract) [102]. Interestingly, the highest levels of beta-glucuronidase activity were seen in rabbits fed a high fat diet (32% calories from fat, including 10% from lard), without blackcurrant extract supplementation, suggesting that dietary fat may also alter enzyme activity [102].

Inhibition of UGT enzymatic activity may be a consideration for modulation of hormone levels and the risk of certain cancers, such as prostate cancer [84]. *In vitro* studies suggest that various foods and food-based components may inhibit UGT activity, including green and black tea, quercetin, rutin, naringenin, allspice, peppermint oil, cacao, and silymarin [84], although further research is needed to evaluate their *in vivo* and clinical effects.

2.2.2. Sulfotransferases. As the name of this superfamily of enzymes might suggest, SULTs are responsible for the transfer of a sulfuryl group donated by 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to hydroxyl or amine groups, particularly in the areas of liver, intestine, adrenal gland, brain, and skin tissues [103]. This process is often referred to as sulfation but is more accurately termed sulfonation or sulfurylation. Decreased function of these enzymes, through genetic variability or presence of environmental chemicals, can lead to eventual interference with thyroid hormone, estrogen, and androgen levels [104, 105], as well as variable polyphenol effects [106], since the active forms of these compounds can be degraded via sulfonation. Typically, once compounds have been conjugated with sulfate, there is less reactivity and toxicity incurred from the precursor molecule [105].

Few *in vivo* studies have examined the effects of dietary components on SULT activity, although caffeine and retinoic acid are possible SULT inducers according to animal studies [107, 108] (Table 6(a)). Although it is uncertain how their outcomes will translate *in vivo*, various *in vitro* studies have indicated the possibility of sulfotransferase inhibition (including competitive inhibition) by wine anthocyanins and flavonols, synthetic food colors (especially red colors), apple and grape juice, catechins including epigallocatechin gallate, quercetin, curcumin, resveratrol, flavonoids (apigenin, chrysin, fisetin, galangin, kaempferol, quercetin, myricetin, naringenin, and naringin), and certain phytoestrogens (daidzein, genistein) [3, 105]. Pyridoxal-6-phosphate, the active form of vitamin B6 (which is widely distributed in foods), may also be a competitive SULT inhibitor, according to one *in vitro* study [109], although human tissue concentrations and clinical effects

Table 5: (a) Human and *in vivo* example nutrient inducers of UGT enzymes. (b) Selected dietary sources of D-glucaric acid.

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
UGTs	Cruciferous vegetables	Clinical	Approximately 5 and 10 servings/d of cruciferous vegetables including frozen broccoli, cauliflower, fresh cabbage (red and green), and fresh radish sprouts [90] 250 g/d each of Brussel sprouts and broccoli [25] 2 oz (56.8 g) watercress three times daily [91]
	Resveratrol *Grapes, wine, peanuts, soy,* and *itadori tea* [32]	Clinical	1 g/d resveratrol [28]: *note high dose used*
	Citrus	Observational	0.5+ servings/day of citrus fruits or foods [92]
	Dandelion	*In vivo*	Free access to 2% dandelion tea solution [53]
	Rooibos tea	*In vivo*	Rooibos tea as sole beverage; concentration 2 g tea leaves/100 mL water steeped for 30 minutes [93]
	Honeybush tea	*In vivo*	Honeybush tea as sole beverage; concentration 4 g tea leaves/100 mL water steeped for 30 minutes [93]
	Rosemary	*In vivo*	Diet of 0.5% rosemary extract [37]
	Soy	*In vivo*	150 and 500 mg/kg soy extract [94]
	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	Diet of 1% ellagic acid [95]
	Ferulic acid *Whole grains, roasted coffee, tomatoes, asparagus, olives, berries, peas, vegetables,* and *citrus* [96]	*In vivo*	Diet of 1% ferulic acid [95]
	Curcumin *Turmeric, curry powder* [34]	*In vivo*	Diet of 1% curcumin [95]
	Astaxanthin *Algae, yeast, salmon, trout, krill, shrimp,* and *crayfish* [38]	*In vivo*	Diets of 0.001–0.03% astaxanthin for 15 days [39]

(b)

Legumes	Mung bean seeds, adzuki bean sprouts [97]
Vegetables and fruits	Oranges, spinach, apples, carrots, alfalfa sprouts, cabbage, Brussel sprouts, cauliflower, broccoli, grapefruit, grapes, peaches, plums, lemons, apricots, sweet cherries, corn, cucumber, lettuce, celery, green pepper, tomato, and potatoes [97–99]

may be vastly different. Of note, caffeic acid demonstrates *in vitro* SULT-inhibitory properties [105]. This finding conflicts with its *in vivo* ability to induce SULT enzymes, as described by Zhou et al. (2012) [107], highlighting the difficulty of extrapolating meaningful conclusions from *in vitro* data.

SULT enzyme activity is dependent on a depletable reserve of inorganic sulfate [112]. Dietary sources of sulfur-containing compounds may therefore play an essential role in SULT function, by providing the substrate for enzyme action (Table 6(b)).

2.2.3. Glutathione S-Transferases. Similar to the aforementioned categories of conjugating enzymes, glutathione S-transferases (GSTs) include a complex of enzymes, whose main function is to attach a glutathione group to a biotransformed metabolite. The production of these enzymes can be induced through the production of reactive oxygen species and via gene transcription involving the antioxidant-responsive element (ARE) and the xenobiotic-responsive element (XRE), which will be subsequently discussed in this paper [113].

Cruciferous and allium vegetables and resveratrol demonstrate ability to induce GSTs in humans [28, 114–117] (Table 7(a)). Observational research also associates citrus consumption with increased GST activity [115]. *In vivo* data also suggest many foods and food constituents to be upregulators of these enzymes, including garlic, fish oil, black soybean, purple sweet potato, curcumin, green tea, rooibos tea, honeybush tea, ellagic acid, rosemary, ghee, and genistein [36, 43, 44, 70, 93, 118–123]. Conjugated linoleic acid has been shown to be at least partly responsible for the effect of ghee [122]. It is possible that the effects of at least some of these foods and bioactive compounds may be due to their upregulation of the Nrf2 signaling pathway.

Table 6: (a) *In vivo* example nutrient inducers of sulfotransferases (SULTs). (b) Selected dietary sources of sulfur-containing compounds (adapted from [110]).

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
SULTs	Caffeine *Coffee, cocoa, black tea,* and *green tea* [111]	*In vivo*	2, 10, and 50 mg/kg caffeine [107]
	Retinoic acid (bioactive form of vitamin A) *Meat (especially liver), fish, egg,* and *dairy products* contain retinol; *apple, apricot, artichokes, arugula, asparagus,* and other plant foods contain provitamin A carotenes [111]	*In vivo*	2, 10, and 50 mg/kg/d retinoic acid suspension in corn oil [108]

(b)

Animal products	Fish, shellfish, lamb, beef, chicken, pork, duck, goose, turkey, egg, and cheese
Legumes	Lentils, peas, and butter beans
Grains	Barley, oatmeal
Vegetables and fruits	Cabbage, horseradish, Brussel sprouts, leeks, cress, haricot beans, apricots, peaches, spinach, and watercress
Nuts and seeds	Brazil nuts, almonds, peanuts, and walnuts
Herbs and spices	Mustard, ginger

Genetic variances, gender, and even possibly body weight appear to play a role in the effects of dietary factors on GST enzymes [114–116]. Clinical investigation of cruciferous and allium vegetables by Lampe et al. (2000) found that an upregulated effect was most marked in women, indicating gender variability, and that the effect was also genotype-dependent, occurring only in GSTM1-null individuals [116]. The same investigators also found that apiaceous vegetables inhibited GST activity, but only in GSTM1+ men [116] (Table 7(b)). High doses of quercetin and genistein have also shown inhibitory effects [123, 126].

There is evidence that at least some of these foods and phytonutrients may exert modulatory rather than absolute inductive/inhibitory effects; Chow et al. (2010) found that resveratrol increased GST only in those with low baseline enzyme levels or activity [28]. It is also noteworthy that bioactive components of crucifers, including isothiocyanates, are substrates for GST enzymes and that GST genotype may therefore alter the response to cruciferous vegetables consumption on other mechanisms such as glutathione peroxidase and superoxide dismutase [134, 135]. GSTM1-null genotype is associated with a more rapid excretion of isothiocyanates, leading some researchers to conclude that the benefits of cruciferous vegetable consumption may be lessened in individuals with this genetic variation [89].

Support for glutathione conjugation also involves enhancing reduced glutathione (GSH) status. Glutathione is a low-molecular weight tripeptide containing residues of cysteine, glutamate, and glycine [136]. Most glutathione from foods and supplements is poorly absorbed, so liposomal delivery has been used [137]. The sulfur-containing amino acids methionine and cystine are important precursors to glutathione formation; their depletion leads to depressed GSH levels [138]. N-acetyl cysteine has also been used to restore depleted GSH levels in a clinical setting [139].

Various nutrients may also enhance endogenous glutathione synthesis, including vitamin B6, magnesium, and selenium [140, 141]. Curcuminoids (from turmeric), silymarin (from milk thistle), folic acid, and alpha-lipoic acid have been shown, in humans, to restore depleted GSH [129, 130, 142, 143]. In animal studies, cruciferous vegetables and artichoke have also demonstrated a GSH-protective effect [131–133]. There is therefore the potential to improve glutathione status via diet or supplementation (Table 7(c)).

2.2.4. Amino Acid Transferases. Amino acids of various types (e.g., taurine, glycine), whether endogenous or exogenous (from dietary sources) in origin, can be utilized for attaching to molecules for their excretion. For the benefit of providing a substrate to these enzymes, it is generally thought that dietary protein is required for an effective detoxification protocol. Table 8 lists amino acids used in phase II conjugation reactions and selected food sources.

2.2.5. N-Acetyl Transferases (NAT). This class of enzymes is responsible for the transfer of an acetyl group to convert aromatic amines or hydrazines to aromatic amides and hydrazides, which is significant for those taking pharmaceuticals such as isoniazid, hydralazine, and sulphonamides [83]. Polymorphisms in genes for this category of enzymes, leading to slow metabolism, have been shown to be associated with hepatoxicity during drug treatment [146]. One small human study found that 500 mg quercetin daily enhanced NAT activity [29]. However, more research is needed to understand the relationship between dietary nutrients and NAT function.

Table 7: (a) *In vivo* example nutrient inducers of glutathione S-transferases (GSTs). (b) *In vivo* example nutrient inhibitors of glutathione S-transferases (GSTs). (c) Selected dietary sources of nutrients for glutathione support ([111] unless otherwise noted).

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
GSTs	Cruciferous vegetables	Clinical, observational	Approximately 5 and 10 servings/d of cruciferous vegetables including frozen broccoli, cauliflower, fresh cabbage (red and green), and fresh radish sprouts [114] >31.2 g/d cruciferous vegetables [115] 4.5 cups of cruciferous vegetables/d, including 0.5 cups of radish sprouts, 1 cup of frozen cauliflower, 2 cups of frozen broccoli, and 1 cup of fresh cabbage [116] 300 g/d cooked Brussels sprouts [117]
	Allium vegetables	Clinical	3 tbsp fresh chives, 1.33 cups of fresh leeks, 1 tsp garlic, and 0.5 cups of fresh onion [116]
	Resveratrol *Grapes, wine, peanuts, soy,* and *itadori tea* [32]	Clinical	1 g/d resveratrol [28]: *note high dose used*
	Citrus	Observational, *in vivo*	>76 g/d citrus [115] 20 mg limonoid mixture every 2 days [124]
	Garlic	*In vivo*	30 to 200 mg/kg garlic oil [36] 80 and 200 mg/kg garlic oil 3 times weekly [70]
	Fish oil	*In vivo*	20.5 g/kg fish oil [36]: *note high dose used*
	Black soybean	*In vivo*	1 g/kg black soybean seed coat extract [44]
	Purple sweet potato	*In vivo*	100 and 200 mg/kg anthocyanin extract from purple sweet potato [118]
	Curcumin	*In vivo*	Diet of 2% curcumin [119]
	Green tea	*In vivo*	Equivalent of 4 cups/d (200 mL each) of green tea [120]
	Rooibos tea	*In vivo*	Rooibos tea as sole beverage; concentration 2 g tea leaves/100 mL water steeped for 30 minutes [93]
	Honeybush tea	*In vivo*	Honeybush tea as sole beverage; concentration 4 g tea leaves/100 mL water steeped for 30 minutes [93]
	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	30 mg/kg/d ellagic acid [43]
	Rosemary	*In vivo*	20 mg/kg carnosic acid 3 times weekly [121]
	Ghee (clarified butter)	*In vivo*	19.5 mg CLA (conjugated linoleic acid)/g fat [122]
	Genistein (kidney GSTs) *Fermented soy* (e.g., *miso, tempeh*) contains up to 40% bioavailable genistein versus 1% or less in other soy products [125]	*In vivo*	1.5 g/kg genistein [123]: *note high dose used*

(b)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
	Apiaceous vegetables	Clinical	1 tsp fresh dill weed, 0.5 cups of fresh celery, 3 tbsp. fresh parsley, 1.25 cups of grated parsnips, and 0.75 cups of frozen carrots [116]

(b) Continued.

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
GSTs	Quercetin *Apple, apricot, blueberries, yellow onion, kale*, and *alfalfa sprouts, green beans, broccoli, black tea*, and *chili powder* [47, 48]	*In vivo*	2 g/kg quercetin [126]: *note high dose used*
	Genistein (liver GSTs) *Fermented soy* (e.g., *miso, tempeh*) containsup to 40% bioavailable genistein, versus 1% or less in other soy products [125]	*In vivo*	1.5 g/kg genistein [123]: *note high dose used*

(c)

Vitamin B6	Turkey, pork, chicken, beef, amaranth, lentils, pistachio nuts, sunflower seeds, garlic, and prunes
Magnesium	Nuts, seeds, beans, and whole grains
Selenium	Brazil nuts, pork, turkey, lamb, chicken, and egg
Methionine	Turkey, pork, chicken, beef, egg, Brazil nuts, soybean, sesame seeds, and spirulina
Cystine	Pork, turkey, chicken, egg, soybean, spirulina, sesame seeds, and oats
Glycine	Turkey, pork, chicken, amaranth, soybean, peanuts, pumpkin seed, and beef
Folate (dietary form of folic acid)	Mung bean, adzuki bean, and other legumes, liver, sunflower seeds, quinoa, spinach, asparagus, avocados, mustard greens, and artichokes
Alpha-lipoic acid	Spinach, broccoli, tomato, peas, Brussels sprouts, and visceral meats [127, 128]
Functional foods	Turmeric, milk thistle, cruciferous vegetables, and artichoke [129–133]

Table 8: Amino acids used in phase II conjugation and selected food sources.

Glycine	Turkey, pork, chicken, soybean, seaweed, eggs, amaranth, beef, mollusks, peanuts, pumpkin seeds, almonds, duck, goose, mung beans, sunflower seeds, lentils, lamb, bison, lobster, and fish [111]
Taurine	Many cooked meats and fish supply taurine. Taurine is also synthesized in the body from cystine (requiring niacin and vitamin B6) and homocysteine (requiring additionally betaine and serine) [144]
Glutamine	Plant and animal proteins such as beef, pork, chicken, dairy products, spinach, parsley, and cabbage [145]
Ornithine	Ornithine is synthesized endogenously via the urea cycle, requiring arginine and magnesium [144]
Arginine	Turkey and pork are especially rich sources; also chicken, pumpkin seeds, soybean, butternuts, egg, peanuts, walnuts, split peas, mollusks, almonds, sesame seeds, lentils, fava beans, mung beans, pine nuts, beef, sunflower seeds, and white beans [111]

2.2.6. Methyltransferases. Relatively significant attention has been given in various medical communities to this class of phase II enzymes due to the increasing importance of methylation for reducing disease risk. The conjugating donor compound in methyltransferase reactions is a methionine group from S-adenosyl-L-methionine (SAMe) [147]. Catechol O-methyltransferase (COMT) is one of the prominent methyltransferases that has received wide attention due to its role in estrogen detoxification [148].

Support for methylation consists of nutrient cofactors and methyl donors, such as methionine, vitamin B12, vitamin B6, betaine, folate, and magnesium [144]. Various foods can provide these nutrients (Table 9). Conversely, a high sucrose diet may inhibit methylation enzymes such as COMT [149].

3. Gene Induction of Phase II Detoxification and Antioxidant Enzymes through Nrf2

The transcription factor, Nrf2 [nuclear factor erythroid 2 (NF-E2) p45-related factor 2], is key to regulating the body's detoxification and antioxidant system. When activated, Nrf2 dissociates from the cytosolic protein, Keap1 (Kelch-like ECH associated protein 1), and translocates to the nucleus to bind to AREs in the promoter/enhancer portion of genes associated with phase II detoxification and antioxidant enzyme genes [150] (Figure 1). Nrf2-deficient animals experience increased toxicity from drugs [151], carcinogens, allergens, and environmental pollutants [152] and do not respond as well to the anti-inflammatory effects of phytochemicals [153], indicating the essentiality of these enzymes. Conversely, Nrf2

Table 9: Selected dietary sources of nutrients for methylation support (adapted from [111]).

Methionine	Meats, poultry, fish, shellfish, egg, nuts (especially Brazil nuts), seeds (especially sesame seeds and pumpkin seeds), spirulina, teff, soybeans Lower amounts found in other legumes and whole grains (especially teff and oats)
Vitamin B12	Meats and meat products (especially liver and kidney), poultry, fish, shellfish, and eggs
Vitamin B6	Meats, nuts (especially pistachio), garlic, whole grains, seeds (especially sesame and sunflower seeds), legumes (especially chickpeas and lentils), and prunes
Betaine	Quinoa, beets, spinach, whole grains (especially rye, kamut, bulgur, amaranth, barley, and oats) sweet potato, meats, and poultry
Folate	Beans and legumes (especially mung beans, adzuki beans, chickpeas, and lentils), liver, nuts (especially peanuts), seeds (especially sunflower seeds), spinach, asparagus, mustard greens, and avocado
Magnesium	Seeds (especially pumpkin seeds and sesame seeds), beans (especially soybeans), nuts (especially Brazil nuts and almonds), and whole grains (especially amaranth)

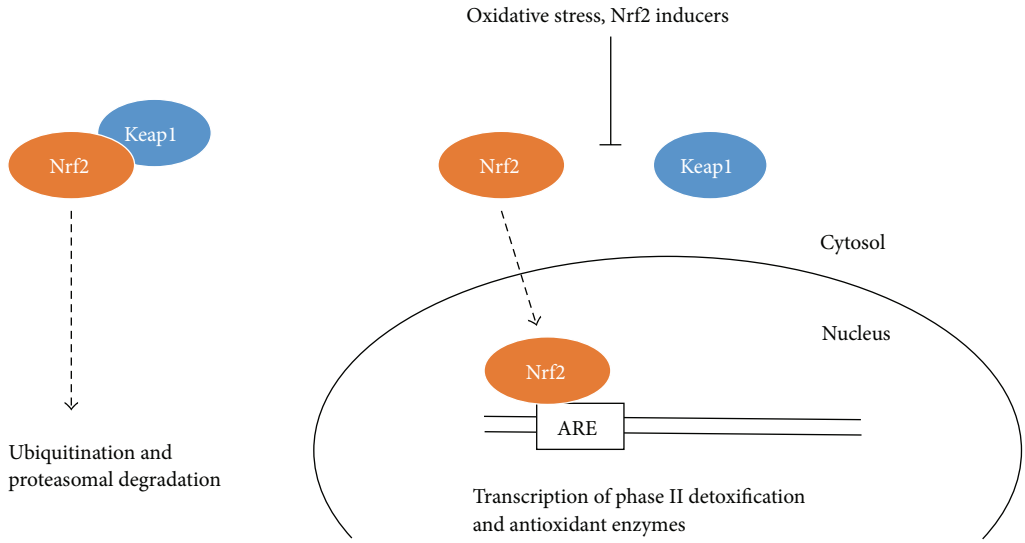

Figure 1: Nrf2/Keap1 signaling (created from text in [154]).

induction is considered protective against various oxidative stress-related conditions such as cancer, kidney dysfunction, pulmonary disorders, arthritis, neurological disease, and cardiovascular disease [154].

Research demonstrates that dietary components, especially phytochemicals, not only scavenge reactive oxygen species, thereby acting as direct antioxidants, but also regulate Nrf2 activity [150]. *In vivo* evidence exists for Nrf2-modulation by curcumin [155–158], broccoli constituents [159, 160], garlic [161–163], epicatechins [164–167], resveratrol [168, 169], ginger [170, 171], purple sweet potato [118], isoflavones [172, 173], coffee [174], rosemary [175, 176], blueberry [166, 177], pomegranate [178], naringenin [179], ellagic acid [166], astaxanthin [166], and γ-tocopherol [180] (Table 10(a)). A clinical trial by Magbanua et al. (2011), investigating the Nrf2 modulation effects of fish oil and lycopene in the context of prostate cancer risk, also demonstrated that these dietary compounds can upregulate Nrf2 signaling and response to oxidative stress in humans [181]. Direct comparison of the magnitude of effect between these compounds can be difficult to gauge. Some information on their relative effects is provided by Kavitha et al. (2013), who ranked the order of potency of the compounds they tested (from highest to lowest) as chlorophyllin (a semisynthetic compound derived from chlorophyll), blueberry, ellagic acid, astaxanthin, and EGCG [166].

Various studies point to the advantageous effects of whole foods, and food combinations, versus specific bioactive compounds. Zhou et al. (2014), for example, illustrate how organosulfur compounds are not the only Nrf2-enhancing bioactive compounds in garlic; garlic carbohydrate derivatives also show Nrf2-modulatory activity [186]. Balstad et al. (2011), in testing the effects of a combination of food extracts on Nrf2 activity in mice, found that the combination produced a larger-than-expected effect, indicating an additive or synergistic effect [176]. By their calculations, the food extract they used equated to a human (70 kg) dose of 14–23 g each of turmeric, rosemary, and thyme, which is clearly not practical for clinical application, as well as 140–233 g each of coffee, red onion, and broccoli. Calabrese et al. (2010) and Houghton et al. (2013) have also argued that Nrf2 inducers exhibit biphasic effects, with lower doses demonstrating stimulatory effects

Table 10: (a) *In vivo* example nutrient inducers of the Nrf2 pathway. (b) *In vivo* example nutrient inhibitors of the Nrf2 pathway.

(a)

Enzyme	Food, beverage, or bioactive compounds *Food sources in italics*	Type of study	Dosages used and references
Nrf2	Fish oil	Clinical	3 × 1 g/d fish oil containing 1098 mg EPA and 549 mg DHA [181]
	Lycopene *Tomatoes, rose hips, guava, watermelon,* and *papaya* [111]	Clinical	2 × 15 mg/d lycopene [181]
	Curcumin *Turmeric, curry powder* [34]	*In vivo*	200 mg/kg/d curcumin [155] 75 mg/kg/d curcumin [156] 50 mg/kg/d curcumin [157] 200 mg/kg/d curcumin [158]
	Cruciferous vegetables	*In vivo*	0.5 mg/kg/d sulforaphane [159] Diet of 15% crushed broccoli seed [160]
	Garlic	*In vivo*	50 and 100 mg/kg/d diallyl disulfide [161] 250 mg/kg/d raw garlic [162] 25 mg/kg S-allyl cysteine [163]
	Catechins *Tea* (especially *green tea*), *cocoa, legumes,* and *grapes* [182]	*In vivo*	5, 15, and 45 mg/kg epicatechin [164] 15 mg/kg epicatechin [165] 20 mg/kg Theaphenon E (95% EGCG) [166] 5, 15, and 30 mg/kg epicatechin [167]
	Resveratrol *Grapes, wine, peanuts, soy,* and *itadori tea* [32]	*In vivo*	10 mg/kg/d [168] 20 mg/kg/d [169]
	Ginger	*In vivo*	100 mg/kg/d [6]-shogaol [170] 10 and 100 mg/kg dried ginger extract [171]
	Purple sweet potato	*In vivo*	100 and 200 mg/kg anthocyanin extract from purple sweet potato [118]
	Isoflavones *Soy, kudzu root,* and *red clover* [183]	*In vivo*	80 mg/kg/d soy isoflavones [172] 60 and 120 mg/kg puerarin from kudzu root [173]
	Coffee	*In vivo*	2.0 mL/d coffee to an average animal weight of 200 g ± 10 g [174]
	Rosemary	*In vivo*	50 and 100 mg/kg carnosic acid [175] 5 mg/animal carnosol extract [176]
	Blueberry	*In vivo*	200 mg/kg blueberry [166] 0.6 and 10 g/day [177]
	Pomegranate	*In vivo*	1 and 10 g/kg pomegranate extract [178]: *note high doses used*
	Naringenin *Citrus* [179]	*In vivo*	50 mg/kg/d naringenin [179]
	Ellagic acid *Berries, pomegranate, grapes, walnuts,* and *blackcurrants* [42]	*In vivo*	Diet of 0.4% ellagic acid [166]
	Asthaxanthin *Algae, yeast, salmon, trout, krill, shrimp,* and *crayfish* [38]	*In vivo*	15 mg/kg astaxanthin [166]
	γ-tocopherol *Nuts, seeds, whole grains, vegetable oils,* and *legumes* [111]	*In vivo*	20.8 mg/kg γ-tocopherol [180]

(b)

Enzyme	Food, beverage, or bioactive compounds	Type of study	Dosages used and references
Nrf2	Luteolin	*In vivo*	40 mg/kg luteolin three times per week [184]
	Quercetin	*In vivo*	50 mg/kg/d quercetin [185]

and higher doses exhibiting Nrf2-interference [187, 188]. These data suggest that the doses found in whole foods may be more beneficial than supplements at supraphysiological doses. In fact, it may well be their weak prooxidant effects that stimulate Nrf2 inducers' favorable antioxidant responses [188].

Nonuniform activities of different foods within the same food group should, once again, be considered; in their recent review of the effects of plant-derived compounds on Nrf2 activation, Stefanson and Bakovic (2014) noted that pak choi, via presumed Nrf2 activation, was more effective at reducing inflammation in the colon than broccoli and that broccoli upregulated some additional Nrf2-related antioxidant enzymes compared with pak choi [189]. Interestingly, this effect was only apparent when steamed, rather than cooked, broccoli was used [189], indicating that food preparation may be an important consideration.

Conversely to its role in cancer prevention, overexpression of Nrf2 is found in many cancer cells and has been shown to promote tumor growth and resistance to anticancer therapy [154]. Consequently, the inhibition of Nrf2 signaling may be clinically relevant for patients receiving cancer chemotherapy [184, 185]. Overexpression of Nrf2 and CYP2E1 has also been associated with impaired GLUT4 activity and insulin resistance [68]. As noted above, supplementation (above levels normally consumed through diet) with certain phytochemicals may have inhibitory effects on Nrf2 activation, including luteolin [184] and quercetin [185] (Table 10(b)). Vitamins A, C, and E and N-acetyl cysteine have also been implicated as Nrf2 inhibitors at high doses [188]. These findings point to the need for further research to clarify outcomes as they relate to specific disease states as well as potential biphasic dose effects.

4. Metallothionein

Metallothionein, a cysteine-rich protein with the ability to bind divalent cations, including toxic metals such as mercury, cadmium, lead, and arsenic, is gaining recognition as an important component in heavy metal detoxification [190–192]. Similar to the upregulation of phase II and antioxidant enzymes, metallothionein can be induced at specific promoter regions of genes by stimuli such as heavy metals, oxidative stress, glucocorticoids, and even zinc [192]. In addition to sequestering heavy metals, it is capable of scavenging free radicals and reducing injury from oxidative stress [192], as well as inhibiting NF-κB signaling [193].

Dietary patterns and nutrients may result in changes in metallothionein production. Lamb et al. (2011) reported a 54% increase in metallothionein mRNA production in a small clinical trial in women with fibromyalgia following an elimination diet in conjunction with a phytonutrient-rich medical food consisting of hops, pomegranate, prune skin, and watercress [194]. Zinc supplementation (15 mg/day) to healthy men over 10 days led to significantly increased metallothionein mRNA, up to 2-fold in leukocytes and up to 4-fold from dried blood spots [195]. Metallothionein has been shown to be decreased in the intestinal mucosa of patients with inflammatory bowel disease (IBD); however, zinc supplementation (300 mg zinc aspartate, equal to 60 mg elemental zinc per day for 4 weeks) in 14 zinc-deficient patients with IBD resulted in slightly higher metallothionein concentration in the intestinal mucosa [196]. Cruciferous phytonutrients may also modulate metallothionein expression, as suggested by a 10-fold increase following a single oral dose of 50 μmol sulforaphane to rats [197]. Chromium may *inhibit* zinc-induced metallothionein expression, according to animal studies by Kimura et al. (2011) [198]. Early-stage, *in vitro* studies also suggest that quercetin and *Cordyceps sinensis*, a mushroom native to the Himalayan region, may upregulate metallothionein expression [199, 200].

5. Clinical Applications

With the continued emergence of data supporting the role of toxins in chronic disease processes, it is becoming increasingly necessary for clinicians to understand how to provide therapeutic modalities to reduce toxin load in patients. In this paper, several studies regarding the influence of foods and food-based nutrients on the systems of detoxification were presented. From the current information presented, listed below are some key concepts for translation into the clinical setting.

5.1. Nonclinical versus Clinical Studies. One of the limitations that comes to the forefront in this collection of studies is how the information, in many cases, is constrained primarily to studies in cells or animals. It remains questionable as to whether similar effects would be seen in humans at moderate, reasonable doses. In the cell studies, it is difficult to anticipate findings due to the lack of pleiotropic activity that occurs in a complex, living system with multiple detoxification systems working simultaneously. Along similar lines, animal studies are often difficult to extrapolate to individuals due to the degree of variability in genotype and environmental phenotype seen in the diverse human population. Therefore, at this time, it is best to take precaution in firmly advocating foods or food-based nutrients that only have cell or animal data as support. It is best to rely on the clinical studies that have been published to date in making more firm recommendations.

5.2. Single Agent versus Lifestyle. While this paper focuses on isolated nutrients and foods that contain those nutrients, it might be optimal from a clinical perspective to consider how an entire lifestyle might induce or inhibit the array of detoxification enzymes. For example, this paper has not addressed behaviors like smoking, physical activity, or stress. The modern clinician needs to weigh all these variables against each other. Yet, science has not fully demonstrated the individual impacts of these factors, along with all of them together. Therefore, at this time, a dietary pattern favoring whole, unprocessed, plant-based foods and the removal or reduction of toxic substances in one's environment is a two-prong approach that would seem to have the best overarching scientific underpinning.

5.3. Modulating versus Inhibiting/Inducing Effects. In several instances, certain foods exhibited a particular activity on an enzyme, while, at higher doses, they had another, opposite effect. Essentially, many foods serve as what is commonly referred to as being "bifunctional modulators," possessing the ability to effectively induce or inhibit detoxification enzyme activity based on the dose response. Therefore, the resulting clinical takeaway might be to encourage patients to follow a mixed, varied diet, full of different plant-based, whole foods. Smaller amounts of many compounds might be more therapeutic and supportive for biochemical pathways rather than overriding signals derived from high concentrations of nutrients through high-dose supplementation or the repeat, daily ingestion of large quantities of the same food.

5.4. Polypharmacy. For patients who are taking multiple pharmaceuticals, it is important to know which detoxification systems will be influenced by nutrients and foods so that side effects are minimized or avoided.

5.5. Dietary Supplements versus Foods. Since there can be potent effects of food-based nutrients on detoxification pathways, it would be best for the average patient to follow, as indicated above, a mixed, complex, and whole-foods diet. Additionally, dietary supplements may be a helpful adjunct in patients in which the practitioner has information about the patient's genetic variability, so that nutrients can be tailored accordingly. Without a full understanding of a patient's SNPs (single nucleotide polymorphisms), it becomes difficult to make accurate assessments about nutrients and dosing.

5.6. Duration of Dosing. Another factor to consider in therapeutic intervention is the timing and duration of the dose of nutrient or the food. In some of the research presented here, effects on detoxification enzymes were seen after several days of food intake or supplementation, while, in other cases, induction of an enzyme might be fairly rapid, followed by efficient adaptability. This variable needs to be considered in further clinical research and requires close monitoring in clinical practice.

5.7. Foods Known to Impact Detoxification. Based on the four systems examined in this paper, there are several foods which seem to have demonstrated an influence on detoxification systems. Many of them have been acknowledged as part of naturopathic medicine. Hence, it would be useful to have a knowledge base of this cumulative set of foods as patients embark upon detoxification protocols. This recent scientific update notes clinical evidence of effects from cruciferous vegetables (in combination, and specifically watercress, garden cress, and broccoli), allium vegetables, apiaceous vegetables, grapefruit, resveratrol, fish oil, quercetin, daidzein, and lycopene. Many other foods, beverages, and nutrient bioactive compounds, based on this review of scientific literature, are also suggested as modulators of detoxification enzymes *in vivo* (Table 11).

Table 11: Food, beverages, and bioactive compounds with demonstrated, or potential, clinical impact on detoxification systems.

Food or beverage	Nutrient bioactive compounds
Allium vegetables	Astaxanthin
Apiaceous vegetables	Caffeic acid
Black raspberry	Catechins (*including EGCG*)
Black tea	Chrysin
Blueberry	Curcumin
Chamomile tea	Daidzein
Chicory root	Ellagic acid
Citrus	Ferulic acid
Coffee	Fish oil
Cruciferous vegetables (*with potential for distinct effects of different crucifers*)	Genistein
	Luteolin
	Lycopene
Dandelion tea	MCTs
Garlic	Myricetin
Ghee	N-acetyl cysteine
Ginger	Naringenin
Grapefruit	Quercetin
Green tea	Resveratrol
Honeybush tea	Retinoic acid (*vitamin A*)
Peppermint tea	
Pomegranate	
Purple sweet potato	
Rooibos tea	
Rosemary	
Soybean/black soybean	
Turmeric	

6. Conclusions

Over the past decade, there has been investigation into nutrigenomic and epigenetic influences of food constituents on chronic diseases [201, 202]. Similarly, studies have revealed that exposure to and accumulation of toxins play a significant role in cardiovascular disease, type 2 diabetes, and obesity [203–207]. Thus, one's dietary intake and environmental influences may have large bearing on the incidence of chronic disease. In fact, these influences may be significant not just for the individual, but for several generations due to the transgenerational inheritance of epigenetic changes [208, 209]. Therefore, it would seem that designing clinical recommendations to maximize the effects of food and reduce the impact of toxins is essential. However, it is not without caution and critical thinking that a detoxification protocol should be assembled for patients by trained clinicians. There remain many unresolved issues regarding knowing how and what foods modulate detoxification pathways.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All authors read and approved the final version of the paper.

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NUTRITION, GENETICS, AND RISKS OF CANCER

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■ **Abstract** Dietary patterns, nutrients, and other constituents of food are major components of the environmental influences that contribute to risk for cancer, and the study of interactions between nutritional and genetic factors is a new and important area of research. This review describes the concepts and principles underlying this area of study and types of relationships between nutritional and genetic factors, and it provides examples of specific diet-gene interactions that are of current interest, with an emphasis on implications for cancer prevention and public health. Polymorphisms exist in the genes for the activating and conjugating metabolizing enzymes, and the induction of metabolizing enzyme activity by nutritional factors may result in either the activation of a carcinogen or the detoxification of a reactive intermediate metabolite. The relationship between the methylenetetrahydrofolate reductase gene and dietary folate is an example of a diet-gene interaction that involves a polymorphism in a vitamin metabolism gene, and the presence of the variant appears to influence both risk for cancer and folate requirements. Diet-gene interactions likely contribute considerably to the observed inter-individual variations in cancer risk in response to exposures to the nutritional factors that have the potential to promote or protect against cancer. Insights into mechanisms by which nutritional factors affect the process of carcinogenesis are provided by knowledge of the targeted gene function and enzyme activity. Increased knowledge in this area will allow a more refined approach to reducing risk for cancer, with diet interventions targeted toward individuals and subgroups that are genetically susceptible and responsive to the effects of nutritional factors.

Investigating the cellular and molecular pathogenesis of cancer is an established and important area of basic science research. Within the past several years, the capability of examining biomarkers of various genetic factors in the development and progression of chronic diseases, such as cancers, has enabled researchers to study these factors in clinical and community-based populations, in which environmental factors can also be measured or even manipulated. Dietary patterns and

the nutrients and other constituents of food are a major component of the environmental influences that appear to contribute to disease risk, and the study of interactions between nutritional and genetic factors is a new and important area of research. This review describes the concepts and principles underlying this area of study, describes types of relationships between nutritional and genetic factors, provides examples of specific diet-gene interactions that are of current interest, and comments on implications for cancer prevention and public health.

BASIC CONCEPTS

Nutrition

Nutrition is the process by which the human body uses food for life, growth, and the normal functioning of every organ and tissue. The link between essential nutrients and health or risk for disease has been well established in this century, but other dietary constituents are now also recognized as having potentially important effects on health and disease risk. These nonnutrient dietary constituents include dietary fiber and phytochemicals, such as allyl sulfides in garlic, indoles in cruciferous vegetables, and other biologically active compounds that are found in foods. Physiologic or metabolic characteristics that are determined at least in part by dietary patterns, such as obesity, are yet another way in which nutrition appears to influence disease risk.

Substantial evidence from epidemiological, clinical, and laboratory studies suggests that nutritional or dietary factors can influence risk for the development of cancer, prognosis after the diagnosis of cancer, and quality of life during cancer treatment. In fact, current estimates are that 20%–60% of cancers in the United States are related to nutritional and dietary factors (3, 23). The molecular pathogenesis of cancer is currently believed to be a multistep process involving the accumulation of genetic changes that result from the interaction between genetics and the environment (28, 41, 63). Notably, genetic factors alone are believed to explain only ∼5% of all cancer (63). Also, several genetic and epigenetic changes contribute to this process, in which normal cellular functions are ultimately lost and clonal expansion of abnormal cells occurs.

Demonstrating the specific causative relationships and underlying mechanisms that link the various dietary constituents to cancer risk has been a challenging task. Typically, nutritional or other environmental factors are associated with only modestly increased relative risks in community-based or population studies. Some of the difficulties in defining the relationships between nutritional factors and disease risk may include limitations in the methodologies for assessing exposure to these factors, difficulties in disentangling the influence of various dietary constituents, and probable interactions between these factors, such as synergistic and additive effects (73, 83). However, it has long been recognized that not all persons exposed to the same risk factors will develop the associated disease (37, 41), and it is now increasingly clear that differential genetic susceptibility may explain

variations in response among individuals with apparently similar diets or exposures to nutritional factors (61).

Recent advances in knowledge of genetics and cellular biology have been facilitated in large part by considerable advances and improvements in molecular technologies, which are now enabling the identification of specific genes and their function. An important initiative that is fueling these advances is the Human Genome Project, currently being carried out by the National Institutes for Health, National Center for Human Genome Research, and the U.S. Department of Energy (17). The major objective of this 15-year project is to map the entire human genome at the base-pair level. The implications of this knowledge for nutritional science and public health research are substantial. In nutrition and cancer research, new methodologies for molecular genetics are now being used in population-based observational and intervention studies, which has resulted in an explosion of interest in potentially important diet-gene interactions. Biomarkers for genetic alterations and activity have been demonstrated to be feasible and useful in many large-scale studies. This area of research is one of rapid growth, as various mutations and variants are identified, and potential interactions between these variants and nutritional factors are being explored (18).

Diet-Gene Interactions

Genetic factors that increase risk for cancer consist of several general types. Probably the best-known are the highly penetrant, dominant mutations that have been associated with very high relative risks in epidemiological studies (80). An example of this type of mutation is breast cancer gene 1 (*BRCA1*). Although this type of genetic mutation is highly penetrant, it is also quite uncommon. *BRCA1* mutations appear to account for 5% of breast cancer cases in women <40 years old, 2% of cases in women aged 40–49 years, and 1% of cases in women aged 50–70 years (79). Although the individual breast cancer risk is substantially increased by the presence of this gene mutation, the public health significance is less clear, because the majority of women who develop breast cancer do not have this or other highly penetrant mutations that have been identified. In fact, recent studies in low-risk populations suggest that many women with *BRCA1* (or *BRCA2*) do not develop breast cancer (48, 79). Other cancers associated with these types of germline gene mutations are familial retinoblastoma, Wilms' tumor, familial polyposis coli, xeroderma pigmentosum A, and a subset of breast and ovarian cancers (Li-Fraumeni syndrome).

Compared with these highly penetrant dominant mutations, genetic mutations that are not sufficient to cause disease but may affect susceptibility to cancer or response to environmental exposures can have a much greater effect on a population level, even though they may pose lower individual risk (63). These types of gene variants are inherited in the population at frequencies that can substantially affect disease rates. A gene variant that occurs in >1% of the population is considered a genetic polymorphism, because a pattern of heritability (rather than chance

DNA mutation) is suggested. The gene variants that influence metabolic activation, detoxification, or elimination of carcinogens have been the most studied to date, and some of these polymorphisms are relatively common in the general population (up to 53%). Genes that influence other aspects of carcinogenesis, such as DNA repair, chromosome instability, the activity of oncogene or tumor suppressor genes, cell cycle control, signal transduction, hormone metabolism, vitamin metabolism pathways, immune function, and receptor action, may also potentially influence susceptibility to dietary and other environmental exposures (80). The nature of the diet-gene interactions that can occur appears to vary widely, as illustrated in the examples provided below.

In studying the biological link between nutritional factors and cancer risk, another aspect of research in diet-gene interactions involves identifying the genetic and epigenetic alterations that constitute the mechanism by which nutrients and other dietary constituents affect the process of carcinogenesis. Epigenetic alterations (i.e. chemical alterations to the DNA that do not involve changes to the coding sequence), although they are not heritable genetic risk factors, can influence cancer risk through modified transcriptional regulation. Knowledge of these DNA alterations is also important for demonstrating a causative relationship between nutritional factors associated with cancer risk in epidemiological studies. For example, folate deficiency in rats has been shown to induce hypomethylation within the *p53* gene, an epigenetic alteration that may be a means by which folate deficiency enhances carcinogenesis (39). Advances in molecular genetics have enabled these mechanistic studies of genetic alterations to be incorporated into clinical and community-based studies involving dietary interventions.

EXAMPLES OF DIET-GENE INTERACTIONS

Overview: Metabolizing Enzymes

Xenobiotic metabolizing enzymes play an important role in activating and/or detoxifying foreign compounds, including carcinogens. Marked inter-individual variability in response to drugs and toxins has been observed. Dietary constituents act as inducing agents through several molecular mechanisms, and many dietary factors (including both micronutrients and nonnutrient constituents of foods) are well-known substrates or inducers of the metabolizing enzyme systems (90). In addition to the recognized dietary inducers, several nutritional factors, such as protein intake, obesity, and fasting, have been shown to influence the activity of the oxidation enzymes in experimental animal studies (25). Polymorphisms exist in the genes for the metabolizing enzymes, and the potential influence of these genetic alterations on risk for cancer, owing to diet-gene interactions, has become the focus of intense research interest.

In the biotransformation of a foreign compound or carcinogen, the first step typically involves the addition of one or more hydroxyl groups to a relatively nonpolar hydrocarbon, which transforms the compound into an electrophilic or more polar

intermediate. These oxidation reactions are carried out by phase I or activating enzymes, the cytochromes P450, which are coded by *CYP* genes. The cytochrome P450 enzymes also catalyze the oxidation of several endogenous compounds, such as steroid hormones and vitamin D metabolites. Phase II or conjugating enzymes catalyze conjugation reactions to compounds such as glutathione, which facilitates elimination. Phase II enzymes include the glutathione *S*-transferases (GSTs), *N*-acetyltransferases (NATs), microsomal epoxide hydrolase, sulfotransferases, and UDP-glucuronosyl-transferases (41). Whether a polymorphic variant of these enzymes increases or decreases risk for cancer depends on the specific enzymatic activity that is being stimulated and the substrate involved. Similarly, the induction of metabolizing enzyme activity by nutritional factors may result in either the activation of a carcinogen or in the detoxification of a reactive intermediate metabolite. Figure 1 illustrates the interrelationships between the biotransformation enzyme systems.

Cytochromes P450 and *CYP* Genes

The cytochromes P450 (and corresponding *CYP* genes) are a superfamily of enzymes responsible for numerous oxidation reactions of endogenous compounds and the phase I activation of a wide range of substrates (25, 82, 90). More than 30 of these enzymes have been characterized in detail, although a relatively small number of P450s appears to be responsible for most xenobiotic oxidation reactions in humans (25). In the general population, polymorphic *CYP* genes result in differences in the ability to oxidize substrates. The P450s have been shown to be induced by chemical carcinogens and various foods and dietary constituents in humans and by micronutrient supplements in laboratory animals (4, 55).

As described above, phase I or activating-enzyme reactions can result in the metabolism of carcinogens to make them more carcinogenic, activated intermediates. For example, P4501A1 activates polycyclic aromatic hydrocarbons (i.e. benzo[*a*]pyrene), and P4501A2 activates heterocyclic amines, which are found in meats cooked at high temperature (80). Also, these substrates and their sources (i.e. tobacco smoke and well-done meat), in addition to cruciferous vegetables (which contain indole-3-carbinol), can induce the activity of P4501A2. The activity of P450s can also be inhibited by dietary constituents such as naringenin, a dietary flavonoid in grapefruit juice (90).

Several studies of the *CYP1A1* gene suggest that polymorphisms in this gene, which occur in ∼10% of the Caucasian population (63), may be an important determinant of cancer risk. Increased lung cancer risk in smokers has been associated with certain *CYP1A1* variants (*MspI*, *exon7* [isoleucine-valine]) (88), and interactions between these variants and conjugating enzyme polymorphisms have been observed. As reviewed by Perera (63), the degree of increased risk associated with *CYP1A1* polymorphism ranges from twofold in heavy smokers to a sevenfold increase in light smokers, when compared with equivalent exposures in smokers without the genotype. For example, the presence of at least one copy of the *CYP1A1 MspI* variant allele was associated with a 2.4-fold increase [95%

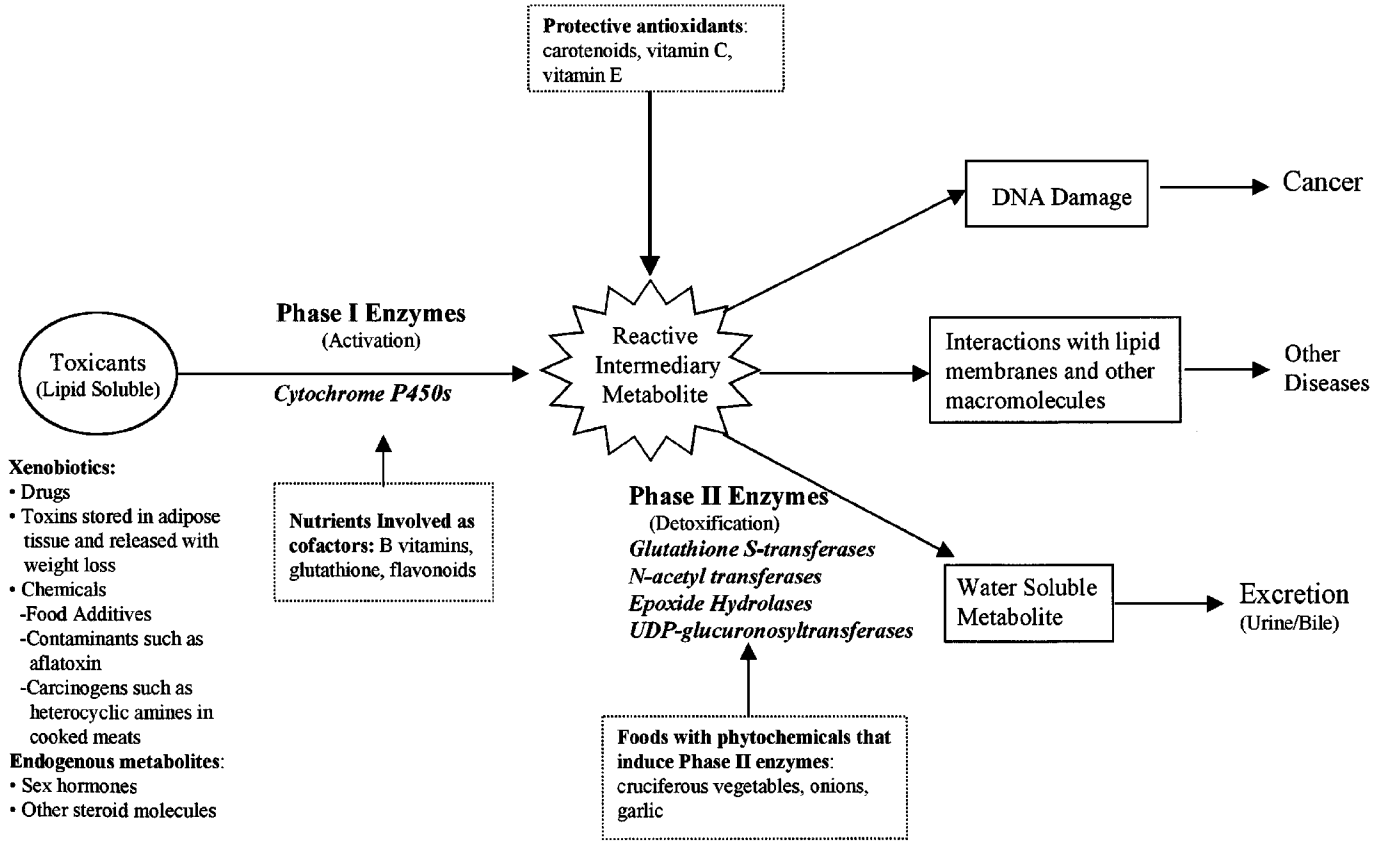

Figure 1 Interrelationships between the biotransformation enzyme systems (reprinted with permission from reference 61).

confidence interval (CI), 1.2–4.7] in the risk for squamous cell carcinoma, and a 3.1-fold increase (95% CI, 1.2–7.9) when combined with a *GSTM1* deletion, adjusted for smoking and dietary variables, in a recent multiethnic case control study (42). Although a significant relationship between the *CYP1A1 MspI* variant and overall lung cancer was not observed in this study, homozygous *CYP2E1 RsaI* and *DraI* genotypes were associated with a 10-fold decrease (95% CI, 0.0–0.5) in overall lung cancer and adenocarcinoma risk. More polycyclic aromatic hydrocarbon-DNA adducts have been found in the leukocytes of smokers with the *exon7* variant compared with those without this variant, and the adduct concentration of lung tissue has been found to correlate with *CYP1A1* expression or enzyme activity (54). Furthermore, mutations in the tumor suppressor gene *p53* have also been linked with the *CYP1A1* genotype in the lung tumors of Japanese smokers (35).

Effects of dietary factors on the endogenous substrates of P450s may also be relevant to the prevention of certain cancers. Indole-3-carbinol, which is found in vegetables such as cauliflower, cabbage, and Brussels sprouts, has been shown to increase estradiol 2-hydroxylase activity in animals and humans (44, 90), owing to the induction of *CYP1A1*. In mice, feeding indole-3-carbinol at doses ranging from 34 to 700 mg/kg/day can promote a fivefold increase in activity of estradiol 2-hydroxylase (11). In a small study of 25 obese and nonobese women, oral doses of 400 mg/day of purified indole-3-carbinol for 2 months increased the ratio of urinary 2-hydroxyestrone to estriol, an indirect measure of 2-hydroxylation activity in the competitive estrogen metabolic pathway, by 82%–93% (53). 2-hydroxylation converts estradiol to less potent metabolites, and it is possible that an increase in this metabolic activity may reduce the risk for estrogen-related cancers, although further studies are clearly needed to demonstrate whether the effect is clinically significant.

The surprising results from two large clinical trials in which supplementation with beta carotene and/or vitamin A was associated with an increased risk for lung cancer in smokers (1, 59) may even be related to an interaction between these micronutrients and cytochromes P450. Liver P450 enzymes have been shown to metabolize retinol and retinoic acid to polar metabolites (68, 72), and the administration of vitamin A, canthaxanthin (a non-provitamin A carotenoid), or a beta-carotene metabolite (β-apo-8′-carotenal) has been shown to induce P450s in laboratory animals (5, 22, 55). In one recent study, *CYP1A1*, *CYP1A2*, and other corresponding P450 genes, which would predispose an individual to cancer risk from the widely bioactivated tobacco smoke procarcinogens (i.e. benzo[*a*]pyrene), were shown to be induced by high doses of beta carotene (500 mg/kg body weight) administered to rats (60). In ferrets fed 2.4 mg/kg/day of beta carotene (calculated to be equivalent to an intake of 30 mg/day of beta carotene in a 70-kg human), exposure to smoke promoted increased oxidation of beta carotene to an abundance of metabolites that are structurally similar to retinoids (87), including β-apo-8′-carotenal, a strong inducer of liver cytochromes P4501A1 and P4501A2 in rats (22). In the study by Wang et al (87), the concentration of retinoic acid in the lung tissue of the smoke-exposed and/or beta carotene-supplemented ferrets was decreased significantly, supporting the suggestion that oxidation products of

beta carotene promoted induction of cytochrome P450 enzymes, which depleted retinoic acid and thus interfered with normal retinoid signaling. At present, no studies in humans that address the effect of carotenoids on P450 enzyme activity have been reported.

Glutathione *S*-Transferases

The GSTs are a major family of cytosolic enzymes that catalyze the conjugation of reduced glutathione to a large number of electrophilic compounds formed by cytochrome P450 enzymes. Because electrophiles can bind to DNA, forming adducts and potentially DNA mutations, GSTs play a critical role in protecting cells against the cytotoxic and mutagenic effects of these reactive compounds. Thus, it has been hypothesized that GST induction results in an overall decreased cancer susceptibility (8) and that, conversely, impaired detoxification by GST will confer increased susceptibility to disease (31).

The GSTs are divided into four major classes—alpha (GSTA), pi (GSTP), mu (GSTM), and theta (GSTT), based on their physicochemical and immunologic properties (49), and, within each class, several isozymes exist. GSTs have been found in all human tissues studied, but with striking differences in isozyme distribution in different tissues and organs (reviewed in 16, 85). For example, of the total hepatic GST protein, ∼80% is GSTA, 10%–20% is GSTM (in *GSTM1*-positive individuals), and <5% is GSTP (76, 86). In the lung, GSTP is the predominant class (85%), whereas GSTM and GSTA isozymes constitute only ∼7%–8% each (86).

GST isozymes have overlapping substrate specificities; however, the GSTA family conjugates predominantly organic hydroperoxides, some steroids, prostaglandins, and alkenals, whereas GSTM conjugates predominantly epoxides and quinones (10, 36, 50) and polycyclic aromatic hydrocarbons, common carcinogens found in tobacco smoke, food, and combustion fumes (64). *GSTT1* metabolizes epoxybutanes, monohalomethanes, and certain alkyl halides (30).

Genetic polymorphisms have been identified in the GSTM, GSTT, and GSTP classes (27, 29, 62, 78). The most intensely studied to date are *GSTM1* and *GSTT1*, for which inherited homozygous deletions result in deficiencies of these two isozymes. The frequency of the *GSTM1* null genotype varies significantly among ethnic populations. For example, it ranges from 22% to 35% among Africans and African-Americans to 38% to 67% in Caucasians, and is apparently 100% among the inhabitants of the Republic of Kiribati (Oceania) (69), which may account in part for some of the international differences in cancer rates. The frequency of the *GSTT1*-null genotype is estimated to be similar to that of the *GSTM1*-null genotype in Africans and Asians, but to be ≤20% among Caucasians (69). Both *GSTM1*- and *GSTT1*-null genotypes, either alone or in combination, confer a higher risk for several types of cancer, particularly among subgroups exposed to sources of carcinogens (e.g. tobacco smoke) (reviewed in 13, 31, 69). At the same time, a number of studies show no effects of the *GSTM1* genotype on risk, suggesting that the *GSTM1*-null genotype is a susceptibility factor of moderate strength (13).

However, in combination with polymorphisms in *CYP* genes (e.g. *CYP1A1* variants that result in increased P4501A1 activity), the *GSTM1* null genotype may be a more important risk factor. For example, among Japanese, the risk of lung cancer was significantly increased in *GSTM1*-null individuals who had the *CYP1A1* (*MspI m2/m2*) [odds ratio (OR), 8.3; 95% CI, 1.44–49.7) or (*m1/m2*) genotype (OR, 5.2; 95% CI, 1.20–22.7), but not the wild type (*m1/m1*) (OR, 2.3; 95% CI, 0.39–12.6) (38). In non-Japanese populations, these gene-gene interactions are weaker, although similar relationships have been reported (31).

Dietary modulation of GSTs has been reported in animal studies and some controlled feeding studies in humans. Types of dietary fiber and fat have been shown to affect hepatic GST activity in rats (70, 74). Numerous phytochemicals, including those in cruciferous vegetables and allium vegetables, increase GSTs in rat liver and other tissues (71, 84, 91). In humans, addition of 300 g of Brussels sprouts (at the expense of 300 g of glucosinolate-free vegetables) increased plasma GSTA concentrations by a factor of 1.4–1.5 in men, without a concomitant change in plasma GSTP concentrations (57). In another feeding study, vegetable juice consumption was associated with an average twofold increase in peripheral lymphocyte GSTP expression among a subset of responders (65); it has been suggested that the cancer-protective effect of vegetables may be dependent on the ability to induce high levels of tissue GSTP. Cruciferous vegetables may have dual or opposing effects, in that the constituents of these foods can both induce *CYP1A2* (which could potentially activate procarcinogens) and also induce *GSTM1* (which could potentially detoxify carcinogens). In fact, an interactive effect of *GSTM1* genotype and cruciferous vegetable intake on *CYP1A2* activity was observed in one recent observational study. *GSTM1*-null individuals exhibited a 21% higher *CYP1A2* activity than *GSTM1*-positive individuals consuming comparable amounts of cruciferous vegetables ($P = 0.01$) (67), presumably owing to higher excretion rates of the vegetable *CYP1A2* inducers in the *GSTM1*-positive participants.

More recently, investigators have begun to examine the differential protective effect of dietary patterns on the basis of *GST* genotypes. For example, in a study of the association between colonic adenomas and cruciferous vegetables, only individuals who were *GSTM1* null received protection from ingestion of large amounts of broccoli (*P* for trend, 0.001; *P* for interaction, 0.01) (43). Furthermore, in *GSTM1*-null smokers, Grinberg-Funes et al (24) reported an inverse relationship between polycyclic aromatic hydrocarbon-DNA adducts and serum concentrations of vitamins E and C ($r = -0.4$; $P < 0.06$). These studies suggest that intakes of antioxidants and dietary constituents that induce GSTs are more important in individuals who lack the *GSTM1* detoxification mechanism and who are exposed to high levels of polycyclic aromatic hydrocarbons.

Diet Interactions with Other Metabolizing Enzymes

Among the other metabolizing enzymes that could be influenced by dietary factors, *NAT* gene interactions with diet have been examined in several epidemiological studies. Two NAT enzymes have been identified in humans, NAT1 and NAT2, and

both have been shown to be polymorphic. These enzymes catalyze the metabolic activation of aromatic and heterocyclic amine carcinogens. A NAT2 phenotype described as a slow acetylator occurs in 50%–60% of Caucasians and 30%–40% of African-Americans (63). Consumption of meat prepared at high temperature, a source of heterocyclic amines and polycyclic aromatic hydrocarbons, and exposure to tobacco smoke have been observed to interact with the *NAT2* genetic variant type and thus may influence risk for colorectal and breast cancer, respectively (80, 92), although the results from studies of the relationship between NAT2 and breast cancer risk have been somewhat inconsistent (2). NAT1 has been suggested to be of greater etiological importance in breast cancer, because NAT1 but not NAT2 activity is detectable in human mammary epithelial cells. In a nested case-control study of postmenopausal Iowa women, Zheng et al (92) found a 30% increased risk (95% CI, 0.8–1.9) for breast cancer associated with the *NAT1*10* allele and a nearly fourfold increased risk (95% CI, 1.5–10.5) associated with the *NAT1*11* allele. The increased risk associated with the *NAT1*11* allele was most evident among smokers (OR, 13.2; 95% CI, 1.5–116) and among those who were in the highest red meat consumption group (OR, 6.1; 95% CI, 1.1–33.2). Similarly, individuals with the rapid NAT2 acetylator type have been observed to be at increased risk of colorectal cancer in association with higher levels of meat consumption, in some (15, 89) but not all (34) epidemiological studies.

Interactions between nutritional factors and several other metabolizing enzymes are also under study. For example, risk for liver cancer in association with exposure to aflatoxin, a fungal contaminant of peanuts, may be modified by the presence of genetic variants of cytochromes P4503A3 and P4503A4 and the phase II enzyme epoxide hydrolase (41, 52). It has been suggested that genetic factors may contribute to risk for cancer in response to aflatoxin exposure because aflatoxin B1 is activated and conjugated via phases I and II enzymes. Aflatoxin B1 is metabolized by the cytochrome P450 enzymes to several products, at least one of which (8,9-exo-epoxide) appears to be mutagenic (26). The aflatoxin B1 epoxide metabolite reacts with DNA as a mutagenic metabolite, but alternatively can be detoxified by epoxide hydrolase. Although the clinical significance has not yet been demonstrated, a role for epoxide hydrolase polymorphisms in determining risk is supported by an observed association between mutant alleles of epoxide hydrolase, aflatoxin B1 adducts, and primary hepatocellular carcinoma (52). Given the importance of metabolizing enzymes in the activation and elimination of potential carcinogens and the demonstrated ability of dietary constituents to influence expression of these genes, additional diet-gene interactions of this type are likely to be identified in population-based studies.

Polymorphisms of Methylenetetrahydrofolate Reductase and Folate

The relationship between the gene encoding methylenetetrahydrofolate reductase (MTHFR) and dietary folate is an example of a diet-gene interaction that involves a polymorphism in a vitamin metabolism gene. The presence of the variant appears to

influence both risk for cancer and folate requirements. A relatively common genetic alteration in the gene encoding MTHFR is the autosomal recessive *C677T* mutation to generate a *val/val* genotype (6, 19), which results in lower specific activity and increased thermolability of the enzyme. Individuals who are homozygous are reported as having 30% of normal enzyme activity, whereas heterozygotes have 65% of normal enzyme activity (19).

The metabolic consequences of this genetic polymorphism are evident in the distribution of folate and related metabolites in the body pools, because MTHFR converts 5,10-methylenetetrahydrofolate, the major intracellular form of folate, to 5-methyltetrahydrofolate, the major form of folate in the circulation. The presence of the MTHFR gene variant is associated with decreased plasma folate and increased plasma homocysteine concentrations, reflecting a shift in the folate metabolic pathway (6, 19), and the presence of this polymorphism has been observed to relate to differences in homocysteine and folate responses to folic acid supplements (6, 56). Plasma homocysteine is a sensitive indicator of folate status owing to folate coenzyme activity in the metabolism of homocysteine to methionine (7). An interaction between the *C677T* MTHFR mutation and plasma folate and homocysteine concentrations has been observed in several studies. Among subjects with lower plasma folate concentrations ($<$15.4 nmol/L), those with the homozygous *C677T* mutant genotype have been observed to have total plasma homocysteine concentrations that are 24% greater ($P < 0.05$) than those of individuals with the normal genotypes (33). In contrast, such differences are not observed between genotype subgroups among individuals with higher folate levels (i.e. $\geq$15.4 nmol/L). Studies involving supplemental folic acid, administered at levels ranging from 0.5 to 5.7 mg/day, suggest that a 25% reduction in homocysteine concentration can be anticipated as a result of increased folate intake (47). Thus, it has been suggested that individuals with the homozygous *C677T* MTHFR genotype have an increased requirement for folate to maintain optimal body pools.

The frequency of the *C677T* polymorphism varies markedly across racial and ethnic groups. In a recent U.S. study (32), the prevalence of several gene mutations, including MTHFR *C677T*, was assessed by polymerase chain reaction in individuals belonging to six racial/ethnic groups. MTHFR *C677T* allele frequencies were higher among Koreans (47%), Hispanics (42%), Native Americans (29%), and Caucasians (27%), compared with African-Americans (12%) and Asian Indians (10%). In U.S. studies involving comparisons between African-Americans and whites (20, 21), homozygosity for the *C677T* genotype among African-American adults has been observed to be very rare. For example, analysis of blood samples in Ohio suggested the allele frequency of *C677T* to be 30% of Caucasians and 10% of African-Americans (51). Overall, rates among whites are 10%–12% for homozygous and up to 50% for those who are heterozygous for the MTHFR *C677T* polymorphism (6), with wide differences in these figures across the different U.S. racial/ethnic minority groups.

Folate plays a crucial role in DNA synthesis and methylation, functions that are biologically relevant to carcinogenesis. Epidemiological studies have linked

low folate status or low levels of intake with increased risk for colorectal, lung, and cervical cancer (58, 66, 77), as well as cardiovascular diseases (9, 40, 47), and abnormalities of DNA methylation in colonic neoplasms are frequently observed (46). In the assessment of the relationship between folate intake, cancer risk, and MTHFR polymorphisms, dietary factors that may influence folate requirements, such as methyl donors and alcohol (which adversely affects folate metabolism), also need to be examined. Before the fortification of all cereal grain products (effective in January 1998), high-folate diets in the United States were basically diets high in vegetables and fruits, and dietary folate quantified in earlier studies may be a marker of a high-vegetable and high-fruit diet (similar to beta carotene), which would provide numerous potentially anticarcinogenic constituents.

In several case control studies (14, 81), intakes of folate and the dietary factors that influence folate status or methylation capabilities (i.e. alcohol and dietary methionine) have been found to modify the pattern of risk for colorectal cancer that is typically associated with MTHFR polymorphisms. For example, the *C677T* variant form of MTHFR was associated with a lower risk of colon cancer than the other types [i.e. RR 0.46 (95% CI, 0.25–0.84) in the Physicians' Health Study] (45), but individuals with this reduced enzyme activity appear to benefit the most (with regard to risk for colon cancer) from a diet high in folate and methionine and low in alcohol (14). Possibly, individuals with the *C677T* variant form of MTHFR may have reduced risk for colorectal cancer because normal DNA synthesis would be promoted by the altered folate metabolic pathway, but they would also be more likely to exhibit difficulty maintaining normal DNA methylation with a more limited supply of folate supply or increased stress on methylation capabilities caused by alcohol intake (14, 33).

SUMMARY AND CONCLUSIONS

Identifying the influence of nutritional factors on risk for cancer is substantially refined by increased knowledge and research on the various genetic polymorphisms, as illustrated in the types of interactions and specific examples described above. If only a subgroup of individuals is sensitive to these factors, the effect may be diluted and thus undetectable when the entire population is the focus of study. Continued research in this area will also help to clarify the biological and biochemical roles of nutritional factors, because insights into mechanisms are provided by knowledge of the targeted gene function and enzyme activity.

With increased knowledge of susceptibility based on genetic factors, diet interventions to prevent cancer can be better targeted to individuals most likely to benefit by diet modification, based on their degree of risk caused by genetic variability. Historically, nutritional guidelines to prevent diseases such as cancer are developed for and aimed to the general population, involving much debate and discussion of priorities because the resources to support these nutrition efforts are necessarily limited. Some of these guidelines and communications are likely to

be of benefit to the majority of individuals regardless of genetic risk factors for cancer, owing to their association with risk for several chronic diseases, such as the recommendation to maintain healthy body weight. However, current evidence from research on diet-gene interactions suggests that some subgroups are more susceptible than others to the increased risk for cancer associated with a dietary pattern, and targeting efforts toward these individuals would be a more efficient use of the limited resources that are available.

There are also substantial limitations and constraints in this area of study. Depending on the prevalence of the genetic polymorphism, the size of the sample needed for sufficient statistical power to examine diet-gene interactions may be an important limitation (75). Also, the difficulties involved in accurately identifying and characterizing exposures to nutritional factors remain daunting in the epidemiological studies in which genetic factors are examined, as in other epidemiological studies, and even the best methods for nutritional and dietary assessment used in population-based research have well-known limitations and weaknesses (12, 73). Given the apparent importance of phytochemicals (as compared with essential nutrients) in many of these interactions, it is important that knowledge of the bioavailability and other pharmacokinetic properties of most of these compounds is quite limited, and, in most cases, a food content database that would allow quantification of intakes of these compounds is simply not available. Many of the potentially influencing nutritional factors are unmeasured in these types of studies, so the risk of confounding or misidentification of surrogate variables as important determinants is high. Also, biomarkers and biological assays that are used to characterize susceptibility of individuals based on apparent genetic variation must have demonstrated specificity and be validated in the targeted populations, or the associations will be based on assumptions that may not be supportable.

Even at this early stage of research, evidence suggests that diet-gene interactions likely contribute considerably to the observed inter-individual variations in cancer risk in response to exposures to the nutritional factors that have the potential to promote or protect against cancer. Genetic factors, in relation to nutritional factors, are sometimes incorrectly perceived as an alternative explanation for cancer risk. In contrast, current evidence suggests that these factors are inextricably linked. Continued research efforts in this area are expected to improve our capability of reducing risk for cancer by diet modifications, with a more refined approach involving targeted nutrition interventions.

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CONTENTS

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APPLIED NUTRITIONAL SCIENCE REPORTS

Nutritional Influences on Estrogen Metabolism

ABSTRACT: *It is now well known that one of the most prominent causes of breast cancer, as well as many other hormone related health problems in both men and women, is excessive estrogen exposure from both endogenous and exogenous sources. Improving estrogen metabolism can be of benefit in women with various conditions and family histories, including a family history of breast, uterine, or ovarian cancer, and conditions such as endometriosis, premenstrual syndrome, uterine fibroid tumors, fibrocystic or painful breasts, cervical dysplasia, and systemic lupus erythematosis. Beneficial modulation of estrogen metabolism can be accomplished through dietary and lifestyle modifications such as increasing fiber and reducing fat, increasing phytoestrogen intake, losing weight, and increasing exercise. In addition, many nutrients effectively reduce estrogen load by supporting preferred pathways of estrogen metabolism and detoxification. These include isoflavones, indole-3-carbinol, B vitamins, magnesium, limonene, calcium D-glucarate, and antioxidants. The influences of these nutrients on estrogen metabolism may have profound significance for diseases and conditions in which estrogen plays a role in clinical expression.*

ESTROGEN PRODUCTION

The term "estrogen" is used to collectively describe the female hormones, the most potent of which is *estradiol*. The other important—but less powerful—estrogens are *estrone* and *estriol*. Estrogens affect the growth, differentiation, and function of diverse target tissues throughout the body—not just those involved in the reproductive process. Estrogens play an important role in bone formation and maintenance, exert cardioprotective effects, and influence behavior and mood. Estrogens also have important actions in male tissues, such as the prostate and testes.[1,2]

In women, estrogens are synthesized from cholesterol in the ovaries in response to pituitary hormones. In an adult woman with normal cycles, the ovarian follicle secretes 70 to 500 µg of estradiol per day, depending on the phase of the menstrual cycle. Estradiol can be converted to estrone and vice versa, and both can be converted to estriol, the major urinary metabolite. Estrogens are also produced by the aromatization of androgens in fat cells, skin, bone, and other tissues.

After menopause, most endogenous estrogen is produced in the peripheral tissues by the conversion of androstenedione, which is secreted by the adrenal cortex, to estrone. In addition, some estrogen continues to be manufactured by aromatase in body fat, and the ovaries continue to produce small amounts of the male hormone testosterone, which is converted to estradiol. The total estrogen produced after menopause, however, is far less than that produced during a woman's reproductive years.[1,2]

Estrogens circulate in the body bound mainly to the sex hormone binding globulin (SHBG); however, only unbound estrogens can enter target-tissue cells and induce biological activity.[1,2] This is an important point, because it means that any change in the concentration of SHBG will alter estrogen metabolism by inducing changes in the availability of estrogen to the target cell.

ESTROGEN METABOLISM AND DETOXIFICATION

Metabolism of estrogen within the body is a complex subject (Figure 1). Estrone and estradiol are biochemically interconvertible and yield the same family of estrogen metabolites as shown for estrone in Figure 1. Because these metabolites vary greatly in biological activity, the ultimate biologic effect of estrogen depends on how it is metabolized. The metabolism of estrogen takes place primarily in the liver through Phase I (hydroxylation) and Phase II (methylation, glucuronidation, and sulfation) pathways, with final excretion in the urine and feces.[1]

- ***Hydroxylation***

Cytochrome P-450 enzymes mediate the hydroxylation of estradiol and estrone, which is the major Phase I metabolic pathway for endogenous estrogens. This takes place at two primary sites on the estrogen molecule, either at the 2 carbon (C-2) position yielding 2-hydroxyestrone (2-OH) or at the 16α carbon (C-16α) position yielding 16α-OH. A minor contribution is made from hydroxylation at the 4 carbon (C-4) position yielding 4-OH.[3] The 2-OH metabolite confers very weak estrogenic activity, and is generally termed the "good" estrogen. In contrast, the 16α-OH and 4-OH metabolites show persistent estrogenic activity and promote tissue proliferation.[3-6] It is suggested that women who metabolize a larger proportion of their endogenous estrogen via the C-16α hydroxylation pathway may be at significantly elevated risk of breast cancer compared with women who metabolize proportionally more estrogen via the C-2 pathway.[3-5,7-9] Furthermore, it is theorized that shifting estrogen balance toward a less estrogenic state through promo-

tion of the C-2 pathway may prove beneficial for a variety of conditions related to estrogen dominance or imbalance.

- ***Methylation***

The 2-OH and 4-OH metabolites (catechol estrogens) are readily oxidized to quinones, which are highly reactive and can damage DNA and promote carcinogenesis directly or indirectly through the generation of reactive oxygen species (ROS). This harmful pathway can be minimized through detoxification and excretion of the catechol estrogens via Phase II methylation by the catechol-*O*-methyltransferase (COMT) enzyme.[5,10,11] This methylation requires S-adenosylmethionine (SAM) and magnesium as cofactors.[11] COMT is present in most tissues and converts catechols into their corresponding methyl ester metabolites, which are more water soluble.[5,7] Recent data suggest that the methylation of 4-OH renders this harmful metabolite significantly less active, while 2-methoxyestrone may manifest beneficial properties by inhibiting breast cancer.[10,12] Therefore, supporting the methylation pathways promotes detoxification of estrogens and provides for more beneficial metabolites of estrogen.

- ***Glucuronidation***

Glucuronidation is one of the key Phase II liver detoxification pathways for estrogens and other toxins. Glucuronic acid is conjugated with the estrogen to facilitate its elimination from the body.[1] Unfortunately, some intestinal bacteria (mostly pathogenic) possess an enzyme, β-glucuronidase, that uncouples the bond between excreted estrogen and glucuronic acid in the large intestine, allowing the estrogen to reenter circulation (enterohepatic recirculation).[13] Not surprising is the finding that excess β-glucuronidase activity is associated with an increased cancer risk, including breast cancer.[14] The activity of β-glucuronidase is increased when the diet is high in fat and low in fiber, and can be reduced by establishing a proper bacterial flora by eating a diet high in plant foods and supplementing the diet with the "friendly bacteria" *Lactobacillus acidophilus* and *Bifidobacterium infantis*.[15]

ESTROGEN RECEPTORS

Estrogens, like all steroid hormones, have a wide range of actions and affect almost all systems in the body, yet act in a tissue-specific manner. Estrogens act by binding with high affinity to the estrogen receptor (ER) in target cells. Once bound by estrogens, the receptor activates the transcription of estrogen-responsive target genes.[16,17] Because the ER has a unique ability to bind with a wide variety of compounds with diverse structural features, many environmental toxins and plant compounds can bind to the ER with varying affinities and modulate estrogen activity.[17]

Two forms of the estrogen receptor, α and β, have been identified that differ in tissue distribution, binding affinity, and biological function.[16,17] Therefore, different target cells may respond differently to the same estrogenic stimulus depending on the ratio of expression of the two receptor subtypes in the cell.[16,17] This helps to explain how phytoestrogens and the new designer estrogen drugs such as tamoxifen and raloxifene—called selective estrogen receptor modulators (SERMs)—behave like estrogens in some tissues but block its action in others. Unraveling the detailed physiological role of each receptor subtype is needed to further elucidate the complex nature of estrogen's mechanisms of action.

ESTROGEN AND CANCER RISK

Epidemiological and animal studies have identified estrogen exposure as a risk factor for several cancers, namely breast, endometrium, ovary, prostate, testis, and thyroid. Much of the evidence comes from the observation that cancer risk increases with increased exposure to endogenous or exogenous estrogens, and the positive relationship observed between blood levels of estrogens and cancer risk.[7,18-22] Prolonged estrogen exposure can cause direct genotoxic effects by inducing cell proliferation in estrogen-dependent target cells (increasing the opportunity for the accumulation of random genetic errors), affecting cellular differentiation, and altering gene expression. Additionally, there is increasing evidence for indirect genotoxic effects of estrogens as well. The relative importance of each mechanism is likely a function of the specific estrogen, as well as the exposed tissue or cell type and its metabolic state.[5,7]

Direct Genotoxic Effects

Evidence is accumulating that some estrogen metabolites may be directly responsible for the initial genetic damage leading to tumors. 16α-OH and 4-OH are the primary estrogen metabolites that have been associated with direct genotoxic effects and carcinogenicity.[5,7] Some researchers believe increased levels of 16α-OH may increase the risk of breast cancer by increasing both cell proliferation and direct DNA damage; however, scientific consensus has not yet been reached.[5,7-9,23] Conversely, 2-OH may induce apoptosis and thereby inhibit cell proliferation, an important mechanism in the prevention of cancer.[12]

A recent 5-year prospective study of 10,786 women was conducted to investigate the role of estrogen metabolism as a predictor of breast cancer, specifically the ratio of 2-OH to 16α-OH.[4] The researchers found that premenopausal women who developed breast cancer had a decreased 2-OH:16α-OH ratio and a higher percentage of 16α-OH than 2-OH. Women with predominately 2-OH were 40% less likely to have developed breast cancer during the 5 years. Another recent case-control study that began in 1977 found that postmenopausal women who developed breast cancer had a 15% lower 2-OH:16α-OH ratio than control subjects.[8] Furthermore, those with the highest 2-OH:16α-OH ratios had about a 30% lower risk to breast cancer than women with lower ratios.

Diverse factors can add to the hormonal risk by decreasing the 2-OH:16α-OH ratio, including numerous pesticides and carcinogens, certain drugs such as cyclosporin and cimetidine (Tagamet), obesity, and genetic predisposition.[24-27] Dietary interventions such as increased consumption of cruciferous vegetables (e.g., broccoli and cabbage) and phytoestrogen-rich foods such as soy and flaxseeds can significantly promote C-2 hydroxylation and increase the 2-OH:16α-OH ratio.

Indirect Genotoxic Effects

Excessive production of ROS has been reported in breast cancer tissue, and free-radical toxicity, which manifests as DNA single-strand breaks, lipid peroxidation, and chromosomal abnormalities, has been reported in hamsters treated with estradiol.[7] The oxidation of catechol estrogens (2-OH and 4-OH) yields reactive

molecules called quinones. Quinones are thought to play a role in carcinogenesis by inducing DNA damage directly or as a result of redox cycling between the quinones and their semiquinone radicals, which generates ROS.[5,7,10] Supplementation with antioxidant nutrients can reduce the oxidation of the catechols and promote greater excretion of these metabolites through the methylation pathway.

RISK FACTORS FOR INCREASED ESTROGEN EXPOSURE

There are many lifestyle factors that can influence the body's production of estrogen. Obesity increases endogenous estrogen production by fat tissue, where the enzyme aromatase converts androgens into estrogen.[18,28] Excess insulin in the bloodstream prompts the ovaries to secrete excess testosterone and reduces SHBG levels, thus increasing levels of free estrogen.[28] Alcohol consumption increases estrogen levels, and epidemiological studies suggest that moderate alcohol consumption increases the risk of breast cancer, an effect that may be synergistically enhanced when combined with estrogen replacement therapy.[29,30]

Two major sources of exogenous estrogens are oral contraceptives and hormone replacement therapy. Another major source is environmental toxins found in pesticides, herbicides, plastics, refrigerants, and industrial solvents that are structurally similar to estrogen and have the ability to mimic harmful estrogens in the body.[17,31] Furthermore, the hormones used to fatten livestock and promote milk production are found in meat and milk products, thereby increasing one's exposure to environmental estrogens.[31]

While these lifestyle and environmental factors do influence the lifetime hormone burden of an individual, endogenous hormone levels also have a genetic basis that can be an important risk factor for hormone-dependent cancers and other conditions. Thus, family history can be a valuable indicator of potential problems in this area. All sources of estrogens—whether environmental, dietary, or endogenously produced—can affect ER function (Table 1). These substances can bind to estrogen α or β receptors with varying affinities and for varying lengths of time, producing a wide range of estrogen-related effects.[17]

Table 1. Sources of Estrogens

Environmental Estrogens[31]	Dietary Estrogens[32-35] ("Phytoestrogens")	Endogenous Estrogens
• Organochlorine chemicals such as vinyl chlorides, dioxins, PCBs, and perchloroethylene (~half of "endocrine disrupters" are in this class) • Aromatic hydrocarbons, phthalates and phenols, and some surfactants • Medications such as hormone replacement, oral contraceptives, tamoxifen, and cimetidine • Hormones in animal products consumed by humans	• Isoflavones (e.g., genistein, daidzein, equol, puerarin, coumestrol, glycitein, biochanins) from soy, beans, peas, clover, alfalfa, and kudzu • Lignans (e.g., matairesinol, pinoresinol, secoisolariciresinol) especially from flaxseed, rye, wheat, and sea vegetables • Certain flavonoids (e.g., rutin, naringenin, luteolin, resveratrol, quercetin) especially from citrus fruits and grapes	• Estradiol • Estrone • Estriol • Hydroxylated estrogen metabolites • Methoxylated estrogen metabolites • Other estrogen metabolites

MANIFESTATIONS OF EXCESSIVE ESTROGEN EXPOSURE AND ESTROGEN DOMINANCE

An abundance of evidence makes it clear that excessive estrogen exposure from both endogenous and exogenous sources is a causal factor in the development of cancer in hormone-dependent tissues, such as the breast, endometrium, ovary, uterus, and prostate. Furthermore, hormonal imbalances between progesterone, testosterone, and estrogen can lead to symptoms and conditions of estrogen dominance. These include premenstrual syndrome (PMS), endometriosis, uterine fibroid tumors, fibrocystic or painful breasts, cervical dysplasia, and systemic lupus erythematosis.

NUTRITIONAL MODULATION OF ESTROGEN METABOLISM

Multiple dietary and nutritional factors have the ability to influence estrogen synthesis and receptor activity, as well as the detoxification pathways through which estrogens are metabolized (Table 2; Figure 1). Incorporating dietary changes with the use of select nutritional supplements can have profound effects in beneficially influencing estrogen balance and thus preventing estrogen-related diseases and conditions. A weight management program may also be very helpful in both reducing adipose aromatase activity and facilitating more desirable estrogen metabolism and excretion.

Dietary Fiber and Lignin

Insoluble dietary fibers such as lignin (found in flaxseeds and the bran layer of grains, beans, and seeds) can interrupt the enterohepatic circulation of estrogens in two ways, thus promoting their excretion and making them less available for reabsorption and further metabolism.[36] First, dietary fiber, especially lignin, can bind unconjugated estrogens in the digestive tract, which are then excreted in the feces. Second, dietary fiber can beneficially affect the composition of intestinal bacteria and reduce intestinal β-glucuronidase activity, resulting in a lowered deconjugation of estrogen and reduced reabsorption.[37] Dietary fiber intake also increases serum concentrations of SHBG, thus reducing levels of free estradiol.[38]

Carbohydrates/Fats/Protein

Complex carbohydrates, such as those found in vegetables and whole grains, are preferred over simple carbohydrates for

optimizing estrogen metabolism. Excess consumption of simple carbohydrates raises blood glucose and insulin levels, resulting in adverse influences on sex hormone balance. Conversely, complex carbohydrates attenuate glycemic and insulinemic responses.[28]

The types and amounts of dietary fats may play a role in determining balance among estrogens in the body. For instance, high-fat diets may promote C-16α hydroxylation over C-2 hydroxylation.[39] Furthermore, omega-3 fatty acids such as eicosapentaenoic acid (EPA) have been shown to increase C-2 hydroxylation and decrease C-16α hydroxylation of estradiol in breast cancer cells.[24]

Inadequate dietary protein may lead to decreases in overall cytochrome P450 activity, including cytochrome P450-1A2, which detoxifies estradiol.[40] Rice fortified with lysine and threonine is a source of protein frequently used to nutritionally support hepatic detoxification function, because of its low allergy potential.[41] Soy is also an excellent source of protein that is low in fat and provides the health benefits of isoflavones.

Phytoestrogens

Phytoestrogens are plant compounds that have the capacity to bind to ERs and appear to have both estrogenic and anti-estrogenic effects, depending on the expression of ER subtypes in target cells and on the level of endogenous estrogen present.[16,17,42] They are currently being extensively investigated as a potential alternative therapy for a range of conditions associated with estrogen imbalance including menopausal symptoms, PMS, and endometriosis, as well as prevention of breast and prostate cancer and protection against cardiovascular disease and osteoporosis.[17,42-44] The two main classes of phytoestrogens are the isoflavones and lignans.

Phytoestrogens beneficially influence estrogen synthesis and metabolism through a variety of mechanisms: 1.) they have a similar structure to estradiol and can bind to the ER,[16,17,43] 2.) they increase plasma SHBG levels,[45] 3.) they decrease aromatase activity,[46] and 4.) they shift estrogen metabolism away from the C-16α pathway to the C-2 pathway.[47,48]

Therefore, it may be possible to demonstrate significant hormonal effects through dietary modification. For example, two recent studies found that increased isoflavone consumption decreased urinary excretion of the genotoxic estrogen metabolites 16α-OH and 4-OH, indicative of their decreased formation, and significantly increased the 2-OH:16α-OH ratio in both pre- and postmenopausal women.[47,48]

Isoflavones—Soy is perhaps the most common food source of isoflavones, but others include legumes, alfalfa, clover, licorice root, and kudzu root. There are several biologically active isoflavones, such as genistein, daidzein, and puerarin, with each plant source delivering a different profile. Higher intakes of soy products and isoflavones, such as consumed in traditional Japanese diets, are associated with low rates of hormone-dependent cancers.[49] The average daily isoflavone intake of Japanese women is 20 to 80 mg, while that of American women is 1 to 3 mg.[50]

In two human studies, women given isoflavone supplements and soymilk for one month experienced longer menstrual cycles and lower serum estradiol levels.[51,52] Longer menstrual cycles are beneficial because they result in decreased lifetime exposure to estrogen and lower the risk for breast cancer. Furthermore, in women with low levels of SHBG, consumption of a soymilk powder providing about 69 mg of isoflavones daily substantially increased their SHBG concentrations, an effect not observed in women with higher initial SHBG levels.[45]

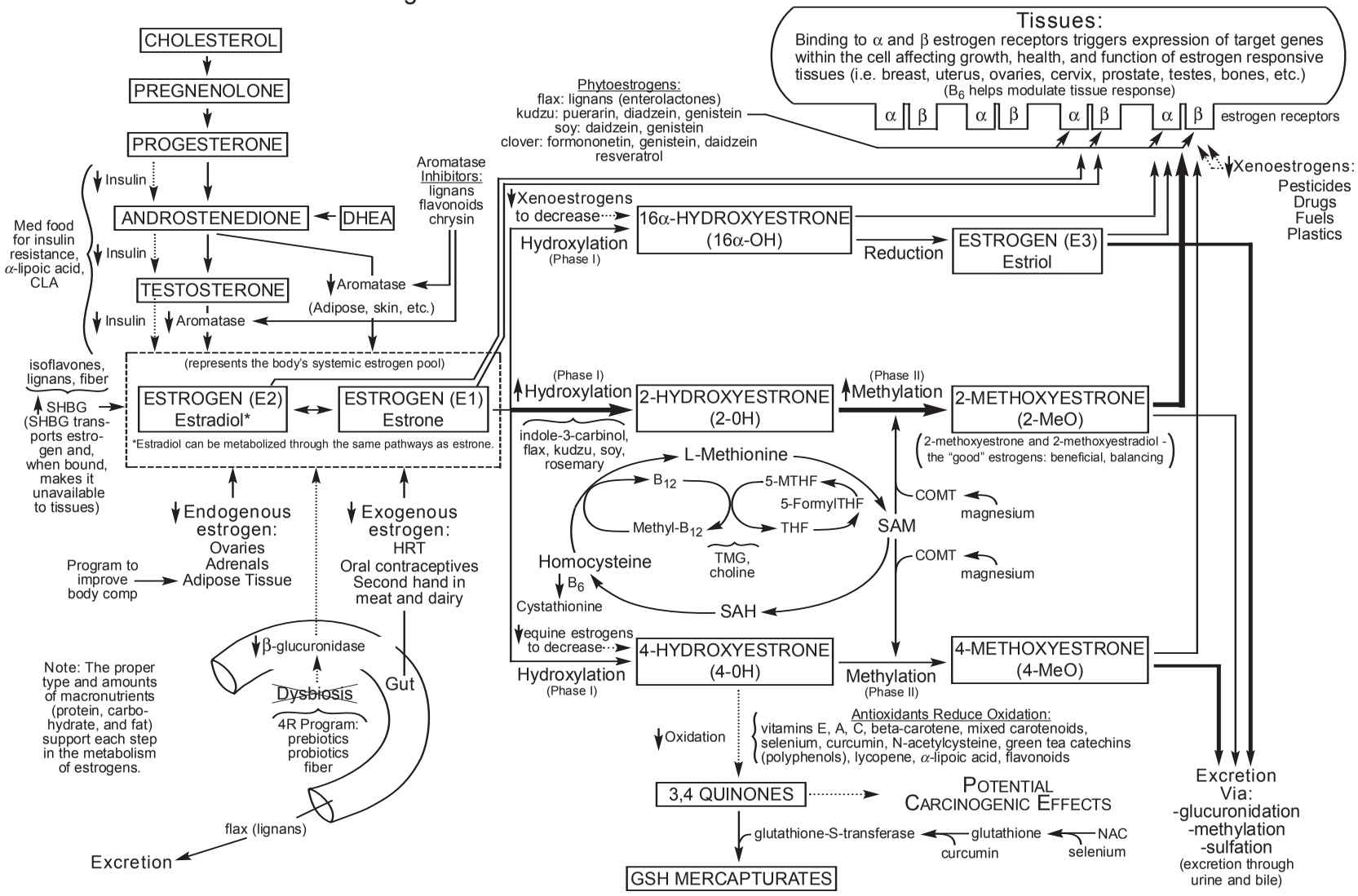
Lignans—These compounds are found in fiber-rich foods such as flaxseed and other oil seeds, whole grains, legumes, and vegetables.[53,54] Lignans stimulate the production of SHBG in the liver, and therefore reduce the levels of free estrogen in circulation. They also inhibit aromatase activity, thus decreasing the conversion of testosterone and androstenedione into estrogens in fat and breast cells.[38,46,55] Lignans also have been shown to inhibit estrogen-sensitive breast cancer cell proliferation.[56] Women consuming 10g of flaxseed per day experienced longer menstrual cycle length, increased progesterone-to-estrogen ratios, and fewer anovulatory cycles, all of which were considered to reflect improved ovarian function.[57]

Table 2. Mechanisms through which dietary and nutritional factors may influence estrogen metabolism

Mechanism of Action	Nutrient
Promote C-2 hydroxylation over C-4 and/or C-16α hydroxylation of estrogens	Cruciferous vegetables, indole-3-carbinol, rosemary, isoflavones (soy, kudzu, clover)
Reduce the oxidation of catechol estrogens (2-OH and 4-OH)	Vitamins A, E, & C, N-acetylcysteine, turmeric, green tea, lycopene, α-lipoic acid, flavonoids
Promote the methylation of catechol estrogens (2-OH and 4-OH)	Folate, vitamins B_2, B_6, & B_{12}, trimethylglycine, magnesium
Increase circulating concentrations of SHBG, thus reducing levels of unbound, active estrogens	Fiber, lignans (flaxseed), isoflavones (soy, kudzu, clover)
Inhibit the activity of aromatase, which converts into estrogens	Lignans (flaxseed), flavonoids (chrysin)
Promote the detoxification of estrogens by upregulating Phase I and Phase II enzymes	Turmeric (curcumin), D-limonene, magnesium, vitamins B_2, B_6, & B_{12}, flavonoids
Inhibit the activity of β-glucuronidase, which deconjugates estrogens in the large intestine, allowing them to be reabsorbed and re-metabolized	Fiber, probiotics (acidophilus, bifidobacteria), calcium D-glucarate
Modify estrogen receptor activity	Isoflavones (soy, kudzu), lignans (flaxseed), indole-3-carbinol, resveratrol

Figure 1.

Nutritional Influences on Estrogen Metabolism



Acronym Key: CLA: conjugated linoleic acid, COMT: catechol-O-methyltransferase, DHEA: dehydroepiandrosterone, 5-FormylTHF: 5-formyltetrahydrofolate, HRT: hormone replacement therapy, 5-MTHF: 5-methyltetrahydrofolate, NAC: N-acetylcysteine, SAM: S-adenosylmethionine, SAH: S-adenosylhomocysteine, SHBG: sex hormone binding globulin, THF: tetrahydrofolate, TMG: trimethylglycine, GSH: glutathione

Resveratrol—This bioflavonoid occurs naturally in grapes and red wine and has been shown to inhibit breast cancer cell growth in vitro.[58] It has been classified as a phytoestrogen based on its ability to bind to and activate the ER,[59] with recent in vitro studies indicating that it exhibits estrogenic and anti-estrogenic activity and binds to ERα and ERβ with comparable affinity.[60,61] These estrogen modulatory effects may explain resveratrol's well-known anticancer and cardioprotective properties.[60]

Vitamin E

Low serum vitamin E is associated with elevated estrogen levels, and supplementation may reduce symptoms of PMS.[62] Vitamin E inhibits growth of breast cancer cells, possibly by inhibiting the expression of vascular endothelial growth factor, which encourages angiogenesis.[63] Furthermore, vitamin E deficiency may negatively affect cytochrome P450 function, thus impacting estrogen detoxification.

Magnesium

Magnesium is an essential cofactor for the COMT enzyme, and therefore optimizes the methylation and excretion of catechol estrogens.[7] Magnesium also promotes estrogen detoxification by directly increasing the activity of glucuronyl transferase, an enzyme involved in hepatic glucuronidation. Ovarian hormones influence magnesium levels, triggering decreases at certain times during the menstrual cycle as well as altering the calcium to magnesium ratio. These cyclical changes can produce many of the well-known symptoms of PMS in women who are deficient in magnesium and/or calcium.[64]

Indole-3-Carbinol (I3C)

I3C is a naturally occurring compound derived from cruciferous vegetables such as broccoli, Brussels sprouts, and cabbage that actively promotes the breakdown of estrogen to the beneficial metabolite, 2-OH. Therefore, I3C is protective to estrogen-sensitive tissues and may be beneficial to those with health issues related to estrogen dominance.

The mechanism by which I3C promotes 2-OH formation involves the selective induction of Phase 1 metabolizing cytochrome P450 enzymes, which facilitate the 2-hydroxylation of estrogen.[65,66] Through this metabolic role, I3C promotes an increased ratio of 2-OH to 16α-OH and may improve estrogen metabolism in women with poor diets or impaired detoxification.[3,65,67] I3C may also reduce the activity of the enzyme required for the 4-hydroxylation of estrogen, thereby decreasing carcinogenic 4-OH formation.[68]

According to a recent human study in both men and women, supplementation with 500 mg and 400 mg of I3C, respectively, resulted in significantly increased urinary excretion of 2-OH, while that of nearly all other metabolites including estradiol and 16α-OH was lower—indicative of their decreased formation.[65] In another double-blind, placebo-controlled study of 57 women at increased risk for breast cancer, supplementation with I3C (300-400 mg/d for 4 weeks) proved to be a promising chemopreventive agent as measured by the increased 2-OH:16α-OH ratio.[69]

Not only does I3C promote healthier estrogen metabolism, but it may also act as a "weak," or anti-estrogen. Through competitive inhibition, I3C has been shown to prevent the receptor binding of "stronger," more stimulating estrogens.[70] Other mechanisms relating to I3C's influence on tissue health involve modulating ER activity, detoxifying xenoestrogens, modulating cell cycle regulation, and preventing the adhesion, migration, and invasion of cancer cell lines.[68,71,72]

B Vitamins

The B vitamins, such as B_6, B_{12}, and folate, function as important cofactors for enzymes involved in estrogen conjugation and methylation. Therefore, decreased levels of B vitamins can disrupt estrogen detoxification and lead to increased levels of circulating estrogens. For instance, folate (as a precursor to SAM) is an essential cofactor for the methylation of catechol estrogens, 2-OH and 4-OH, which reduces their conversion to the carcinogenic quinones.[11] Unfortunately, many individuals have a genetic polymorphism that interferes with their ability to metabolize folic acid to the active form utilized by the body. Supplementing with a metabolically active form of folate that doesn't require enzymatic conversion, such as L-5-methyl tetrahydrofolate, will ensure that these patients maintain adequate folate nutriture.[73]

Another way in which certain B vitamins play a role in estrogen activity is through a potential to modulate the cell's response to activation of the ER. It has been demonstrated that elevated intracellular concentrations of the active form of vitamin B_6 can lead to significantly decreased gene transcription responses when estrogen binds to the ER.[74] By modulating estrogen-induced gene expression in this way, vitamin B_6 can attenuate the biological effects of estrogen. B vitamins also play a role in the prevention of cancer because they are crucial for DNA synthesis and repair as well as the process of DNA methylation, which is essential for DNA stability and integrity and is an important regulator of gene expression.

Calcium D-Glucarate

Calcium D-glucarate is a natural compound that appears to have some influence on breast cancer by aiding in detoxification and the regulation of estrogen.[75] It not only inhibits β-glucuronidase, but also increases the activity of the glucuronidation Phase II pathway, with the net effect of increased estrogen and toxin elimination from the body.[76] Calcium D-glucarate has been found in animal models to lower estradiol levels and inhibit the initiation, promotion, and progression of cancer.[75]

Other Beneficial Phytonutrients

There are many other naturally occurring compounds derived from a variety of plant sources that promote healthy estrogen metabolism. Curcumin is a polyphenol complex from the curry spice turmeric, a member of the ginger family. A combination of curcumin and the isoflavone genistein has shown synergy in reducing xenoestrogen-induced growth of breast cancer cells.[77] Curcumin also increases hepatic levels of glutathione and induces glutathione-S-transferase (GST) and glucuronyl transferase, important in the Phase II detoxification of quinones produced from the oxidation of catechol estrogens.[78,79] Chrysin is a bioflavonoid that has been shown to inhibit aromatase activity, thus reducing the conversion of androgens into estrogen.[80] Aromatase is found in breast tissue, and its inhibition may be useful in reducing the cell proliferative effects of estrogen. Preliminary research indicates that the herb rosemary promotes the 2-hydroxylation of estrogen in a similar fashion to I3C, and

may inhibit 16α hydroxylation. Rosemary may also enhance estrogen detoxification.[81]

Furthermore, many antioxidants and phytonutrients can reduce the oxidation of catechol estrogen metabolites into quinones. Notable players in this group include vitamins E and C, α-lipoic acid, N-acetylcysteine, the mineral selenium, curcumin, and green tea. D-Limonene, a naturally occurring monoterpene found in the oils of citrus fruits, promotes the detoxification of estrogen by inducing Phase I and Phase II enzymes in the liver, including GST.[82] This compound has also shown great promise in the prevention and treatment of breast and other cancers.[83]

There are also many hormone-modulating herbs that have a long history of traditional use in treating women's health conditions, including black cohosh, chasteberry, ginseng, dong quai, and licorice. While the mechanism of action of these herbs varies, many have been found to contain phytoestrogens. For a comprehensive discussion of the use of nutritional supplements and herbs in treating PMS, menopause, and other women's health conditions, please refer to the articles titled, *Premenstrual Syndrome: A Natural Approach to Management; A Healthy Menstrual Cycle; A Natural Approach to Menopause;* and *Black Cohosh and Chasteberry: Herbs Valued by Women for Centuries.*

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Nutritional Influences on Estrogen Metabolism: A Summary

Estrogen affects the growth, differentiation, and function of tissues throughout the body—not just those involved in reproduction. It plays an important role in bone health, protects the cardiovascular system, and influences behavior and mood. While appropriate levels of estrogens are essential for good health, several studies conclude that as exposure to estrogen increases, the risk of several cancers, including breast, ovary, prostate, and thyroid, also increases.[7,18-22] Furthermore, excessive estrogen exposure can lead to other health problems such as premenstrual syndrome (PMS), endometriosis, and fibrocystic or painful breasts.

Various lifestyle and environmental factors can influence estrogen production, metabolism, and balance. These include poor diet, obesity, excess alcohol consumption, high insulin levels, medications such as hormone replacement therapy and birth control pills, overexposure to chemicals found in pesticides and industrial chemicals, and agricultural hormones in animal products consumed by humans.[17,18,28-31] Genetics can also play an important role in determining estrogen levels.

THE BASICS OF ESTROGEN METABOLISM

"Estrogen" is a term that is used to collectively describe the female hormones estradiol, estrone, and estriol. The most potent of these is estradiol. Estrogens circulate in the body mainly bound to the sex hormone binding globulin (SHBG) and only unbound estrogens can enter cells and cause biological effects.[1,2] Therefore, any change in the concentration of SHBG will alter estrogen activity by changing the availability of estrogen to the target cell.

The ultimate biologic effect of estrogen in the body depends on how it is metabolized. The metabolism of estrogen takes place primarily in the liver through Phase I (*hydroxylation*) and Phase II (*methylation* and *glucuronidation*) pathways, which allow the estrogen to be detoxified and excreted from the body. *Hydroxylation* yields 3 metabolites that vary greatly in biological activity: 2-hydroxyestrone (2-OH), 16α-OH, or 4-OH.[3] The 2-OH metabolite is generally termed the "good" estrogen because it generates very weak (and therefore potentially less harmful) estrogenic activity in the body. In contrast, the 16α-OH and 4-OH metabolites show persistent estrogenic activity and may promote dangerous tissue growth.[3-6] In fact, women who metabolize a larger proportion of their estrogen via the 16α-OH metabolite may be at significantly higher risk of developing breast cancer.[3-5,7-9] Therefore, shifting estrogen balance toward a less estrogenic state through promotion of the 2-OH pathway may prove very beneficial in improving a variety of conditions related to elevated or imbalanced estrogen levels.

The 2-OH and 4-OH estrogen metabolites are further detoxified via a process called *methylation*. This is an important pathway, because it renders the harmful 4-OH metabolite significantly less active. Furthermore, if they are not methylated, the 2-OH and 4-OH estrogens can be converted to highly reactive molecules that can damage DNA.[5,10,11] *Glucoronidation* is one of the key Phase II liver detoxification pathways for estrogen, facilitating its elimination from the body.[1]

NUTRITIONAL SUPPORT OF OPTIMUM ESTROGEN METABOLISM

Many elements of good nutrition and diet play an important part in influencing estrogen metabolism and detoxification. Incorporating dietary changes with the addition of beneficial nutrients and herbs can profoundly affect estrogen balance and potentially reduce the risk of estrogen-dependent cancers and other hormone-related conditions.

Diet—It has been found that dietary interventions such as increasing consumption of cruciferous vegetables like cabbage and broccoli, and foods such as soy can significantly increase the 2-hydroxylation of estrogen. Dietary fiber intake can promote the excretion of estrogen by binding estrogens in the digestive tract and also increases SHBG, thus reducing levels of free estradiol.[36,38] Complex carbohydrates, such as those found in vegetables and whole grains, are more effective in optimizing estrogen metabolism than simple carbohydrates, which can raise blood glucose and insulin levels, resulting in secondary adverse influences on sex hormone balance.[28]

Phytoestrogens—These plant compounds are similar in shape to the estrogen molecule and can bind to estrogen receptors (ERs). They are much weaker than endogenous estrogens and, through competitive inhibition, have been shown to prevent the receptor binding of "stronger," more stimulating estrogens.[16,17,42] Phytoestrogens are currently under extensive investigation as a potential alternative therapy for a range of conditions associated with estrogen imbalance, including menopausal symptoms, PMS, endometriosis, prevention of breast and prostate cancer, and protection against heart disease and osteoporosis.[17,42-44]

The two main classes of phytoestrogens are isoflavones and lignans. Soy is perhaps the most common food source of isoflavones, but other excellent sources include legumes, clover, and kudzu root. Higher intakes of soy products and isoflavones, such as consumed in traditional Japanese diets, are associated with low rates of hormone-dependent cancers.[49] Lignans are compounds found in fiber-rich foods such as flaxseeds, whole grains, legumes, and vegetables.[53,54] Lignans stimulate the production of SHBG in the liver, and therefore reduce the levels of free estrogen in circulation. They also inhibit aromatase, an enzyme that synthesizes estrogen.

Vitamin E and Magnesium—Low serum vitamin E is associated with elevated estrogen levels, and may negatively affect estrogen detoxification. Women with PMS have experienced improvements of their symptoms when given supplemental vitamin E.[62] Magnesium promotes estrogen detoxification by promoting methylation and glucuronidation, key estrogen detoxification pathways. Ovarian hormones influence magnesium levels, triggering decreases at certain times during the menstrual cycle as well as altering the calcium to magnesium ratio. These cyclical changes can produce many of the well-known symptoms of PMS in women who are deficient in magnesium and/or calcium.[64]

Indole-3-Carbinol (I3C)—I3C is a naturally occurring compound derived from cruciferous vegetables that actively promotes the breakdown of estrogen via the beneficial 2-OH pathway.[3,65-67] Therefore, I3C is protective to estrogen-sensitive tissues and may be beneficial to those with health issues related to excessive estrogen. Not only does I3C promote healthier estrogen metabolism, but it may also act as a "weak" or anti-estrogen in a similar fashion to isoflavones.[70]

B Vitamins—Folate, B_6, and B_{12} function as important cofactors for enzymes involved in estrogen detoxification; thus, decreased levels of B vitamins can lead to increased levels of circulating estrogens. Certain B vitamins also have the potential to modulate the biological effects of estrogen by decreasing the cell's response when estrogen binds to the ER.[74] B vitamins also play a role in the prevention of cancer because they are important for DNA synthesis and repair.

Calcium D-Glucarate—Calcium D-glucarate is a natural compound found in foods that appears to have some influence on breast cancer by aiding in detoxification and the regulation of estrogen.[75,76] It has been found in animal models to lower estradiol levels and inhibit the initiation, promotion, and progression of cancer.[75]

OTHER BENEFICIAL PHYTONUTRIENTS AND HERBS

Many other compounds derived from a variety of plant sources are available that promote healthy estrogen metabolism. These include curcumin, a compound found in the herb turmeric (*Curcuma longa*) that increases the phase II detoxification of estrogens;[78,79] chrysin, a bioflavonoid that inhibits aromatase activity, thus reducing the synthesis of estrogen;[80] the herb, rosemary, which promotes the formation of the 2-OH estrogen metabolite;[81] and D-limonene from citrus fruits, which promotes the detoxification of estrogen and shows promise in the prevention and treatment of breast and other cancers.[82,83] Furthermore, many antioxidant nutrients and phytonutrients can reduce the oxidation of the 2-OH and 4-OH estrogen metabolites. Notable nutrients in this group include vitamin C, N-acetylcysteine, the mineral selenium, and green tea.

In addition, traditional societies have long relied on a variety of hormone-modulating herbs in treating women's health conditions. These include black cohosh, chasteberry, ginseng, dong quai, and licorice. The mechanism of action of these herbs varies; however, many have been found to contain beneficial phytoestrogens.