Interindividual differences in response to plant-based diets: implications for cancer risk1–4

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ABSTRACT
Genetic differences in taste preference, food tolerance, and phytochemical absorption and metabolism all potentially influence the effect of plant-based diets on cancer risk. Diet is a mixture of carcinogens, mutagens, and protective agents, many of which are metabolized by biotransformation enzymes. Genetic polymorphisms that alter protein expression or enzyme function can modify risk. Genotypes associated with more favorable handling of carcinogens may be associated with less favorable handling of phytochemicals. For example, glutathione S-transferases detoxify polycyclic aromatic hydrocarbons and metabolize isothiocyanates, which are chemopreventive compounds in cruciferous vegetables. A polymorphism in the GSTM1 gene results in lack of GSTM1-1 protein. Pharmacokinetic studies suggest that lack of GSTM1 enzyme is associated with more rapid excretion of the isothiocyanate sulforaphane; therefore, individuals who have this genetic variation may derive less benefit from consuming cruciferous vegetables. Flavonoids are conjugated with glucuronide and sulfate and are excreted in urine and bile. Polymorphisms in UDP-glucuronosyltransferases and sulfotransferases may contribute to variability in phytochemical clearance and efficacy. Genetic polymorphisms in enzymes that metabolize phytochemicals may account in part for variation in disease risk and also have to be considered in the context of other aspects of human genetics, gut bacterial genetics, and environmental exposures. Am J Clin Nutr 2009;89(suppl):1553S–7S.

INTRODUCTION
Phytochemicals are bioactive nonnutrient chemical compounds found in plant foods, eg, fruits, vegetables, grains, nuts, and seeds. They often are categorized into groups on the basis of their chemical structure (ie, polyphenols, organosulfur compounds, carotenoids, alkaloids, and nitrogen-containing compounds). The polyphenols can be divided further into flavonoids (including flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavones), phenolic acids, stiblenes, coumarins, and tannins (1).

Many phytochemicals affect biological processes and have the potential to influence disease risk via complementary mechanisms (1–5). Although animal models of carcinogenesis often show protective effects of plant-food constituents (3, 6), the protective effect of a high plant-food diet in relation to cancer risk in humans has not been established conclusively (7). Human genetic variation may be one of the factors modifying response to constituents of plant foods in population-based studies. This variation may contribute in part to the observed heterogeneity across different study populations. Characterizing how genetic factors modify the effects of plant foods or specific components of these foods may enhance understanding of the potential for high plant-food diets to influence cancer risk and help identify populations that may particularly benefit.

PHYTOCHEMICAL DISPOSITION
Phytochemical disposition, like the disposition of drugs and other xenobiotics, involves absorption, metabolism, distribution, and excretion, and each of these aspects may contribute to variability in exposure (8). Many phytochemicals present in plant foods are glycosides or other conjugates and need to be hydrolyzed to be absorbed (9). Hydrolysis can be carried out by brush border membrane-bound β-glucosidases (eg, lactase phlorizin hydrolase) or by gut bacterial β-glucosidases in the lower small intestine and colon. Once absorbed, aglycones undergo extensive first-pass metabolism in the gut epithelium or liver, with many compounds being conjugated with glutathione, glucuronic acid, or, to a lesser extent, sulfate. Conjugation in the intestinal epithelium and liver by UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) results in conjugates that are excreted in urine and bile. Those that are re-excreted in bile are deconjugated by bacterial β-glucuronidase and can undergo enterohepatic recycling.

The transport of ingested phytochemicals across the intestinal epithelium is another factor determining bioavailability on oral intake. For many phytochemicals and other xenobiotic compounds, transcellular transport is carried out by specific proteins—the membrane-bound, ATP-binding cassette transport proteins, which can translocate a variety of conjugated and unconjugated compounds from the epithelial cells to the submucosal side, facilitating absorption, or back into the intestinal lumen, reducing bioavailability (10). Animal and cell-based studies have shown

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a role for several of the transporters in regulating the uptake of various flavonoids and other phytochemicals (11, 12).

Some phytochemicals undergo phase I, activating reactions in the liver. Several studies have shown, by using human liver microsomes or in vivo in pharmacokinetic studies, that hydroxylation of lignans, isoflavones, and other flavonoids can occur and produces an array of secondary oxidation products (13–17). Oxidation products seem to be minor metabolites of most phenols, however, probably due to rapid conjugation of the would-be phase I substrates in the intestinal epithelium and the liver. In contrast, isothiocyanates (ITCs) derived from cruciferous vegetables have been shown to undergo extensive phase I metabolism in rats (18), although it has not been determined to what extent these reactions occur in humans.

In theory, genetic variation in pathways affecting phytochemical absorption, metabolism, and distribution likely influences exposure at the tissue level (8). Few studies have systematically addressed the factors that contribute to the substantial variation in the metabolism and disposition of phytochemicals in vivo. Expression and activity of many of the enzymes important in phytochemical metabolism are modulated by the substrates they act on and by other xenobiotic compounds (Figure 1). Similarly, genetic variation in the pathways within which of these compounds act can alter biological response. Beyond a few well-recognized conditions (eg, favism: glucose-6-phosphate dehydrogenase deficiency and vicine and covicine ingestion), however, little is known about the biological effects of genetic variation on these gene-phytochemical interactions in humans, especially as they relate to cancer risk. One aspect of phytochemical handling that has received some attention, particularly in relation to specific phytochemicals, is the effect of the genetically polymorphic phase I, conjugating enzymes.

PHYTOCHEMICAL METABOLISM BY POLYMORPHIC PHASE II CONJUGATING ENZYMES

Phytochemicals are conjugated in vivo by biotransformation enzymes in a manner similar to that of other xenobiotics. Many classes of phytochemicals are rapidly conjugated with glutathione, glucuronide, and sulfate moieties and are excreted in urine and bile. Thus, in theory, polymorphisms in biotransformation enzymes, eg, glutathione S-transferases (GSTs), UGTs, and SULTs, have the capacity to affect phytochemical metabolism in the same fashion as they do carcinogens and other xenobiotics. Given that diet is a mixture of carcinogens, mutagens, and protective agents that are all metabolized by the same biotransformation enzymes, genotypes that are associated with more favorable handling of carcinogens (ie, rapid conjugation and clearance of mutagenic substances) may be associated with less favorable handling of phytochemicals (ie, more rapid clearance of potentially beneficial compounds) (see Figure 1). As outlined in Figure 2, genetic polymorphisms that result in proteins with lower enzymatic activity as compared with wild-type protein may result in pharmacokinetic profiles with greater area under the curve (ie, longer exposure), whereas polymorphisms that result in proteins with higher activity may shorten exposure.

Glutathione S-transferases

To date, probably the most studied group of compounds with regard to effects of genetics on metabolism are the ITCs in cruciferous vegetables. The primary route of in vivo metabolism of ITCs is via the mercapturic acid pathway, a major pathway for elimination of many xenobiotics (19). Thiol conjugates of ITCs are formed by conjugation of ITCs with glutathione; this is catalyzed by GSTs. Subsequently, stepwise cleavage of glutamine and glycine yields l-cysteine–ITC, which is acetylated to produce N-acetyl-l-cysteine ITC conjugates (mercapturic acids); these are excreted in urine. Thus, GSTs play an important role in disposition of ITCs in humans. Relationships between cruciferous vegetable intake and cancer risk may be influenced by genetic polymorphisms in biotransformation enzymes that metabolize ITCs and possibly in receptors and transcription factors that interact with these compounds.

In humans there are 3 major GST families: cytosolic GSTs, mitochondrially GSTs, and microsomal GSTs, referred to as membrane-associated proteins in eicosanoid and glutathione metabolism (20). Cytosolic GST are the largest GST family, containing 7 classes: α, ζ, θ, μ, π, σ, and ω GST. Two studies examined the ITC metabolism with different GST isoforms and showed that GSTM1-1 and GSTP1-1 were the most efficient catalysts, GSTA1-1 was less efficient, and GSTM2-2 and GSTM4-4 were the least efficient (21, 22).

Depending on the population, 27–53% of people are homozygous for a common deletion of the GSTM1 gene (GSTM1%), which results in lack of GSTM1 activity. Similarly, 20–47% of people in various ethnic groups are homozygous for a deletion of...
the GSTT1 gene (GSTT1*0) and do not express GSTT1 (23). Investigators have hypothesized that individuals who are null for GSTM1 and GSTT1, and who therefore conjugate and excrete ITCs less readily, would have greater amounts of ITCs at the tissue level and hence experience a greater protective effect of glucosinolate consumption (23). Results of one Chinese population–based study of ITC excretion showed that urinary ITC was higher among GSTT1-positive individuals compared with GSTT1-null individuals but that GSTM1 and Pl genotypes had no effect (24). In contrast, in another Chinese population, Fowke et al (25) evaluated the same GST enzymes and showed a positive association between intake of cruciferous vegetables and urinary ITC excretion only among individuals who had variation in the GSTP1 Ile1/le genotype.

More recently, a pharmacokinetic study of sulforaphane disposition showed that GSTM1-null individuals, compared with GSTM1-positive individuals, had greater areas under the curve for plasma sulforaphane metabolite concentrations, faster rates of urinary sulforaphane metabolite excretion in the first 6 h after consumption, and higher total excretion of sulforaphane and its metabolites over 24 h (26). In a feeding study of a single meal of broccoli (2.5 g broccoli/kg body weight), urinary ITC concentration did not differ by GSTM1, GSTP1, and GSTA1 genotypes except that there was a tendency toward higher ITC excretion in GSTT1-positive individuals. By using a chi-square analysis, the investigators observed a higher proportion of GSTM1-null individuals who had high urinary ITC excretion compared with the proportion of GSTM1-positive individuals who had high urinary ITC excretion (27). These studies do not support the hypothesis that lack of GST enzyme activity reduces clearance of ITCs. Both studies were conducted with a single serving of broccoli. In contrast, more prolonged feeding studies might reflect habitual dietary practices among populations that routinely consume cruciferous vegetables. Whether or not there are differences between acute and chronic feeding or differences in the varieties of ITCs present in broccoli compared with those present in cruciferous vegetables commonly consumed in China remains to be established (26); nonetheless, these results indicate a need to understand further how genotype influences ITC disposition.

Polymorphisms in ITC-metabolizing enzymes may affect response of other biotransformation enzymes to ITC exposure. In a cross-sectional study of frequent broccoli consumers, GSTM1-null individuals had a 21% higher CYP1A2 activity than did GSTM1-positive individuals (28). This result was not observed in a controlled feeding study designed to test a priori the effect of GSTM1 genotype on response to a diet high in cruciferous vegetables; increased CYP1A2 activity in those individuals on the cruciferous vegetable–containing diet was not altered by GSTM1 genotype (29). In the same feeding study, serum GSTx concentration, which is a surrogate measure of hepatic GSTx and an enzyme also induced by ITCs, increased significantly in response to cruciferous vegetable consumption but only in GSTM1-null individuals (30).

UDP-glucuronosyltransferases

UGTs are a superfamily of enzymes that catalyze the addition of glucuronic acid to a range of endogenous and exogenous compounds, including several classes of phytochemicals. The substrate-binding regions of the UGT genes are highly polymorphic and many result in amino acid changes that alter enzyme activity to varying degrees in in vitro systems (31, 32). The functional effect of an alteration in UGT protein often is substrate specific. In vivo, where multiple UGTs are expressed in the same tissue, the overall effect often is less clear. The UGT1A1*28 polymorphism has been associated most strongly with altered xenobiotic glucuronidation in vivo.

Dietary flavonoids are structurally diverse. This group of compounds includes the flavones and flavonols (eg, apigenin, chrysin, galangin, luteolin, and quercetin), flavanes (eg, catechin, hesperetin, and naringenin), and isoflavonoids (eg, genistein and daidzein). The selectivity of glucuronosyl conjugation of the flavonoids is dependent on the structure of a particular flavonoid and on the UGT enzyme involved in its conjugation. For example, UGT1A1, UGT1A8, and UGT1A9 are especially active in conjugating luteolin and quercetin, whereas UGT1A4, UGT1A10, and UGT2B7 and UGT2B15 in the UGT2B family are less efficient (33). The isoflavone genistein is conjugated by UGT1A3, UGT1A8, and, with less efficiency, UGT1A1 and UGT1A10 but not by UGT2B15 (34, 35).

The effects of UGT polymorphisms on flavonoid clearance have not been examined. Studies showing that polymorphisms affect glucuronidation and clearance of drugs and other xenobiotic compounds suggest that it is possible that similar effects may be seen with dietary flavonoids. For example, the UGT1A1*28 polymorphism, which results in 30–40% lower UGT1A1 gene transcription among homozygous variant individuals, is associated with increased toxicity in patients with colorectal cancer treated with the topoisomerase I inhibitor irinotecan (36). In relation to diet, Peters et al also showed that with exposure to well-cooked red meat (a source of mutagenic compounds) in a controlled feeding study, individuals who had the *1/*28 and *28/*28 genotypes had a higher urinary mutagenicity index than did individuals who had the *1/*1 genotypes (37). The authors hypothesized that greater amounts of the mutagens were being excreted in the free form rather than being glucuronidated and deactivated. Furthermore, Chung et al (38) showed that the UGT2B15 *2/*2 genotype group was associated with 42% lower systemic clearance compared with *1/*1 genotype group for lorazepam in healthy volunteers. Although having this polymorphism may result in adverse responses in the context of exposure to toxic compounds or carcinogens, it may be beneficial in the context of reduced conjugation of phytochemicals. This remains to be studied.

Another UGT polymorphism also has been shown to speed drug clearance, but the effect on phytochemical metabolism remains unknown. The enzyme associated with the UGT1A4 (L48V) variant glucuronidates tamoxifen and its active metabolites at a faster rate than the wild-type protein (39). Women at high risk of breast cancer who take tamoxifen as a chemopreventive agent, in particular those who have the UGT1A4 (L48V) polymorphism, may experience reduced effectiveness of antiestrogen therapy (39).

Sulfotransferases

The majority of flavonoid conjugates in circulation or excreted in urine are glucuronides; however, 2–10% also are conjugated with a sulfate moiety by cytosolic SULTs in the liver and gastrointestinal tract. Because sulfated compounds can be deconjugated in target tissues (40), circulating sulfate conjugates of...
phytochemicals may act as a source of tissue aglycones. SULT1A1 has shown high sulfating activity toward a variety of flavonoids, isoflavonoids, and other phenolic dietary compounds, and SULT1A3 has high activity toward some flavonoids but not isoflavonoids (41, 42). Genetic variants in SULT genes with associated functional differences in the translated protein have been identified. Single-nucleotide polymorphisms in SULT1A1 and SULT2A1 are common and have been associated with altered response to therapeutic agents and sex steroid concentrations, respectively [reviewed by Nowell and Falany (43)]. Studies in vitro with recombinant SULT1A1 have shown that the SULT1A1*2 variant is less effective than SULT1A1*1 at conjugating resveratrol, apigenin, and epicatechin (44). Similarly, this could influence the in vivo disposition of phytochemicals metabolized by SULT1A1; however, this remains to be evaluated.

SUMMARY

Dietary intake of a phytochemical or its precursor is not guaranteed to equate with exposure at the tissue level. Several factors may contribute to variation in phytochemical metabolism and disposition, including the following: gut microbial community and activity; genetic determinants of biotransformation enzyme expression, stability, and activity; environmental exposures that influence gut microbiota and biotransformation enzymes; and endogenous constituents that modulate biotransformation pathways. Improved characterization of genetic factors that contribute to interindividual differences in phytochemical disposition may clarify the role of these dietary constituents in cancer prevention. (Other articles in this supplement to the Journal include references 45–71.)

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