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Comparison of short- and long-term exposure effects of cruciferous and apiaceous vegetables on carcinogen metabolizing enzymes in Wistar rats

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ABSTRACT

Cruciferous and apiaceous vegetables may be chemopreventive due to their ability to modulate carcinogen-metabolizing enzymes but whether the effects on such enzymes are sustained over time is unknown. To examine the short- and long-term effects of the vegetables, rats were fed one of four diets for 7, 30, or 60 d: AIN-93G, CRU (21% cruciferous vegetables-fresh broccoli, green cabbage, watercress), API (9% apiaceous vegetables - fresh parsnips, celery), or API + CRU (10.5% CRU + 4.5% API). Although CRU increased activity and protein expression of cytochrome P450 (CYP) 1A1 and CYP1A2 after 7 d, only activity was sustained after 30 and 60 d. There was a trend towards an interaction between the length of feeding period and CRU for CYP1A1 activity; activity increased with greater time of feeding. API increased CYP1A2 activity but decreased sulfotransferase 1A1 activity after 7 d, although not at later times. Altogether, increased CYP1A activity by CRU was maintained with long term feeding while protein amount decreased, suggesting influence by mechanisms other than, or in addition to, transcriptional regulation. Thus, response patterns and interactions with length of feeding may differ, depending upon the types of vegetables and enzymes, requiring caution when interpreting the results of short-term feeding studies.

1. Introduction

A diet rich in fruits and vegetables may be protective against certain cancers (Vieira et al., 2016). One of the mechanisms for this chemopreventive effect may be the ability of certain plant compounds, referred to as phytochemicals, to reduce the mutagenicity of carcinogens. This may be mediated by influencing biotransformation enzymes (Gross-Steinmeyer and Eaton, 2012; Navarro et al., 2011). Specifically, apiaceous vegetables (e.g., celery and parsnips) and certain furanocoumarins therein may be chemopreventive by inhibiting cytochrome P450 (CYP) 1A (Kang et al., 2011; Peterson et al., 2006), as these enzymes activate procarcinogens such as heterocyclic aromatic amines (HAA), benzo[*a*]pyrene, and aflatoxins (Androutsopoulos et al., 2009; Shimada et al., 2001). Interestingly, cruciferous vegetables (e.g., broccoli and cabbage) and the putative chemopreventive metabolites of glucosinolates obtained from crucifers, act as bifunctional inducers. That is, cruciferous vegetables increase not only CYP1As but also carcinogen-conjugating enzymes [e.g., uridine diphosphate glucuronosyl transferase (UGT), glutathione-*S*-transferase (GST)]. These conjugating enzymes increase solubility of carcinogens and facilitate their excretion, thereby potentially decreasing cancer risk (Navarro et al., 2009; Scholl et al., 2011).

Critical roles of biotransformation enzymes in chemoprevention

Abbreviations: AHR, Aryl Hydrocarbon Receptor; AIN, American Institute for Nutrition; API, apiaceous vegetable supplemented diet; CDNB, 1-chloro-2, 4-dinitrobenzene; CRU, cruciferous vegetable supplemented diet; CRU+API, cruciferous and apiaceous vegetable supplemented diet; CYP, cytochrome P450; GST, glutathione *S*-transferase; HAA, heterocyclic aromatic amine; 5-MOP, 5-methoxypsoralen; 8-MOP, 8-methoxypsoralen; SULT, sulfotransferase; UGT, uridine 5′-diphospho-glucuronosyltransferase.

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were also confirmed in multiple human studies in which associations between cancer susceptibility and polymorphic xenobiotic metabolizing enzymes were examined, particularly in the context of HAA metabolism; for instance, a higher activity of CYP1A2 increased urinary mutagenicity after a meal of pan fried hamburger, especially in slow N-acetyltransferase 2 acetylators (Pavanello et al., 2002). Similarly, in a case control study, the *GST1*B/*B* genotype, associated with low protein expression, was associated with an increased risk of colorectal cancer in humans who consume more than two servings of well-done meat per week (Sweeney et al., 2002). These results together indicate that the carcinogenic potency of dietary procarcinogens is strongly dependent upon their biotransformation. Therefore, a clearer understanding of dietary modulation of biotransformation enzymes may allow development of better chemoprevention strategies.

To our knowledge, however, most feeding studies examining dietary modulation of biotransformation enzymes have been short in duration ($\leq$2 wk). Furthermore, the few feeding studies of longer duration have reported reductions in cancer risk markers but found no change in the activity of biotransformation enzymes. For example, in rats exposed to the colon carcinogen 1,2-dimethylhydrazine, both fresh and lyophilized cruciferous vegetable feeding for 15-wk and 9-wk reduced aberrant crypt foci and mucin-depleted foci, precancerous lesions in the colon (Arikawa and Gallaher, 2008); however, CYP2E1, GST, and quinone reductase activities, enzymes involved in metabolizing this carcinogen, were not changed relative to the vegetable-free control group. This suggests that the modulation of biotransformation enzymes observed in short-term feeding studies, such as a 2-wk intervention (Robbins et al., 2010) and a 4-d intervention (Perocco et al., 2006), may not be sustained with chronic vegetable consumption. Moreover, the concept of supplementing with natural compounds to achieve chemoprevention has been criticized. Specifically, Potter addressed that many chemoprevention trials have failed to recapitulate benefits in large population studies; these null effects or even deleterious results from these trials are possibly due to the use of single substances with supra-physiologic doses (Potter, 2014). This underscores the importance of whole-food diet approaches at physiologically relevant levels and warrants having a better understanding of time course effects.

Hence, we investigated whether feeding cruciferous and apiaceous vegetables (individually and combined) would affect activities of the biotransformation enzymes CYP1A1, CYP1A2, sulfotransferase (SULT) 1A1, UGT1A, and GST over time. We chose these specific enzymes due to their roles in activating procarcinogens such as HAA [CYP1A1, CYP1A2, and SULT1A1 (Buonarati et al., 1990; Crofts et al., 1998; Zhao et al., 1994)], their cross-substrate specificity leading to implications for carcinogens other than HAA, and their roles in inactivating procarcinogens and carcinogens through conjugation [UGT1A and GST (Malfatti et al., 2005; Sheweita and Tilmisany, 2003)].

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 225–249 g for the 7 d feeding group and 150–174 g for the 30 d and 60 d feeding groups, were purchased from Harlan Laboratories (Indianapolis, IN, USA). All rats were housed separately in wire-bottom stainless steel cages. Upon arrival, animals were adapted to the basal diet, described below, for 7 d. Animals were allowed free access to water and food. Animal handling and housing followed the NIH guidelines and experimental procedures were approved by the University of Minnesota Animal Care and Use Committee.

2.2. Study diets

The AIN-93G purified diet (Reeves et al., 1993) was used for the basal diet. Organically grown vegetables were purchased from a local market (Minneapolis, MN, USA). The cruciferous supplemented diet (21% wt:wt, CRU) consisted of fresh watercress, broccoli, and green cabbage; each is rich in a different glucosinolate (that give rise to different putative chemopreventive metabolites): gluconasturtiin (phenethyl isothiocyanate), glucoraphanin (sulforaphane), and glucobrassicin (indole-3-carbinol), respectively. The apiaceous vegetable supplemented diet (9% wt:wt, API) included fresh celery and parsnip, which are rich in furanocoumarins with 5-methoxypsoralen and 8-methoxypsoralen commonly detected in them (Lombaert et al., 2001). The combination diet (4.5% wt:wt apiaceous vegetables+10.5% wt:wt cruciferous vegetables, API + CRU) included all aforementioned vegetables. The amounts of vegetables supplemented in the diets were based on a previous report that 22.6% of the same cruciferous vegetables reduced colon cancer risk in rats (Arikawa and Gallaher, 2008). Due to the numbers of vegetables (i.e., broccoli, cabbage, and watercress) to be supplemented, it was decided to supplement 7% of each. In another report, a 7:4 ratio of combined intake of cruciferous and apiaceous vegetables by humans resulted in greater modulation of biotransformation enzymes by apiaceous vegetables over cruciferous (Peterson et al., 2009), leading us to test 9% of apiaceous vegetables here. For the combination group, the diet contained 10.5% cruciferous, and 4.5% apiaceous vegetables.

Vegetables were trimmed, ground, and then added to the basal diet as follows: 70 g of each cruciferous vegetable/kg diet for CRU, 45 g of each apiaceous vegetable/kg diet for API, and 35 g of each cruciferous vegetable plus 22.5 g of each apiaceous vegetable/kg diet for API + CRU. Diets were prepared every 10–13 days and aliquots of each diet were stored in bags at −80 °C. Aliquots were thawed daily and fed to animals. Diet compositions are shown in Table 1. All vegetable-supplemented diets were balanced for macronutrients. Food intakes are expressed on a dry weight basis, using the following formula to convert the fresh weight of vegetables to dry weight:

DW = FW - (Wet weights of vegetables in FW) + (Dry weights of vegetables in FW),

where DW = dry weight of a diet and FW = fresh weight of a diet.

Dry weights for each individual vegetable were taken from the United States Department of Agriculture nutrient database and summed according to diet.

2.3. Experimental design

A total of 121 rats were divided into three groups, each fed their respective diets for different durations of 7, 30, or 60 d. Each feeding time period had a total of 40 rats that were further divided into the four diet groups (n = 10–11 per group): basal, CRU, API, and API + CRU. For randomization, animals were allocated into 10 blocks of 8 rats, 2 blocks of 15 rats, and 1 block of 11 rats.

2.4. Isolation of hepatic microsomes and cytosol

At the end of their assigned feeding duration, animals were fasted for 12 h and then anesthetized with isoflurane. Rats were killed by exsanguination and hepatic microsomes and cytosol were isolated from fresh liver tissue as described elsewhere (Prasad et al., 1985). Both microsomal and cytosolic samples were flushed with nitrogen and then kept at −80 °C until analyzed.