Soy and Whey Proteins Downregulate DMBA-Induced Liver and Mammary Gland CYP1 Expression in Female Rats

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ABSTRACT One possible mechanism by which diet may reduce cancer risk is through enhancement of metabolic systems that prevent activation of carcinogens or accelerate carcinogen inactivation. We studied the effects of diet and 7,12-dimethylbenz-(a)anthracene (DMBA) on hepatic and mammary gland CYP1A1, CYP1A2 and CYP1B1 enzymes in female Sprague-Dawley rats. Diets (AIN-93G) were fed from conception to adulthood, and DMBA was given by oral gavage at age 48–50 d. The protein sources of diets were casein (CAS), soy protein isolate (SPI) or whey protein hydrolysate (WPH). The DMBA-induced hepatic ethoxyresorufin-O-deethylase and methoxyresorufin-O-demethylase activities and CYP1A1 protein and mRNA expression were lower (P < 0.05) in SPI-fed rats compared with those fed CAS. Differences in mammary gland CYP1 expression were also observed with decreased DMBA induction (P < 0.05) of all three CYP1 proteins and mRNAs in rats fed either SPI or WPH compared with those fed CAS. Most notable were the decreased constitutive and DMBA-induced mammary gland expression of CYP1B1 protein of 93 and 96%, respectively, in the SPI-fed rats relative to the CAS-fed controls. The diet-induced changes in CYP1 enzyme expression were consistent with changes in the AhR and ARNT transcription factors that regulate them. Decreased (P < 0.05) mammary constitutive AhR and ARNT proteins were measured in SPI-fed rats. There was also a 100% increase in constitutive CYP1B1 protein in the mammary gland. These results demonstrate the importance of diet in regulation of phase I metabolism in liver and mammary gland, and suggest a potential mechanism by which soy or whey proteins reduce DMBA-induced mammary tumor incidence.


KEY WORDS: ● cytochrome P450 ● rats ● soy protein ● whey protein ● casein.

Diet is considered a risk factor in the development of numerous diseases, including cancer (1). Results of epidemiological studies suggest that consumption of Asian diets that contain high levels of soybean products lowers the incidence of breast, prostate and colon cancers (2,3). In addition, experimental studies have reported that the consumption of bovine milk proteins may also afford cancer prevention (4). We have been studying the mechanism by which soy or whey proteins reduce DMBA-induced mammary tumor incidence. Several of the anticancer properties of soy products have been attributed to phytoestrogens, such as genistein, that are bound to soy proteins. Numerous in vitro studies have revealed that genistein can inhibit the growth of tumor cells and the activities of many enzymes needed for cellular growth and differentiation (6). We have reported that in soy-fed male rats, dexamethasone-induction of hepatic phase I detoxication enzymes, such as CYP3A, was greater than in casein-fed rats (7), and 3-methylcholanthrene induction of CYP1A1, a P450 enzyme involved in carcinogen activation, was reduced in rats fed soy (8). Others have reported that rats fed soy diets had elevated activities of the phase II detoxication enzymes glutathione transferases (GSTs), uridine diphosphate glucuronyl transferase (UDPGT) and NADPH-oxidoreductase (NQO1) (9,10). These soy effects on phase I and phase II enzymes would result in lower tissue concentrations of DMBA and may explain the decreased mammary DMBA-DNA adducts measured in soy- or genistein-fed rats (11).

The anticancer properties of bovine whey protein have been attributed to its ability to elevate cellular glutathione levels, which would also aid in the phase II GST-dependent conjugation of electrophiles, such as bioactivated DMBA (4). Additionally, whey has been reported to enhance the immune system (12) and its anticancer properties may, therefore, be
related to increases in immune surveillance of transformed cells.

DMBA-induced rat mammary carcinoma closely resembles human breast cancer. Similar to those in humans, rat mammary tumors arise from ductal epithelial cells, the occurrence of tumors is preceded by hyperplastic and premalignant lesions, and the histogenesis, morphology and progression of hyperplastic, premalignant and malignant lesions are similar in many respects to those of human breast cancer (13). Thus, DMBA-induced mammary tumors in rats have proven to be a useful tool for studying the molecular mechanisms involved in initiation, progression, pathogenesis and prevention of human breast cancer. This model is especially well suited for studies of diet and cancer risk.

DMBA is a procarcinogen and requires metabolic conversion to its ultimate carcinogenic metabolite, DMBA-3,4-dihydrodiol-1,2-epoxide, a process that includes two separate oxidations by the microsomal CYP1 enzymes. The first oxidation product from Affinity dicyanovinyl (Garden, CO), horseradish peroxidase, CYP1A1 or CYP1B1 (14). The second oxidation produces the highly mutagenic 3,4-dihydrodiol-1,2-epoxide metabolite and is catalyzed by CYP1B1 (15). CYP1A1 and CYP1B1 are expressed in both the liver and the mammary gland and both enzymes are induced by DMBA (15). Thus, the extent to which DNA adducts occur after administration of DMBA depends on the level of oxidative metabolism of DMBA, which in turn is determined in part by the activities of CYP1A1 and CYP1B1.

The purpose of this study was to measure the effects of SPI and WPH on the hepatic and mammary expression of CYP1A1 and CYP1B1 enzymes that are important in the bioactivation of carcinogens such as DMBA. In addition, CYP1A2 was also measured because, like CYP1A1 and CYP1B1, its expression is also regulated by the DMBA-activated aryl hydrocarbon receptor (AhR). Thus, important mechanistic information was gained by studying the diet effects on all three AhR-regulated genes.

### MATERIALS AND METHODS

#### Chemicals and reagents

All chemicals unless otherwise specified were purchased from Sigma Chemical (St. Louis, MO). Resorufin was purchased from Pierce Chemical (Rockford, IL) and methoxyresorufin was purchased from Molecular Probes (Eugene, OR). Radionuclides were purchased from DuPont NEN (Boston, MA). Rabbit polyclonal antibodies against rat CYP1A1/CYP1A2 and CYP1B1 were purchased from Gentest (Woburn, MA); monoclonal antibodies against CYP1A1 and CYP1A2 were purchased from Amersham Pharmacia Biotech (Arlington Heights, IL); goat polyclonal antibody against AhR was purchased from Novus Biologicals (Littleton, CO); and rabbit polyclonal antibody against ARNT was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

#### Animal treatments

The experiments received prior approval from our Institutional Animal Care and Use Committee. Animals were purchased from Harlan Industries (Madison, WI). All animals were housed in an AAALAC-approved animal facility with a 12-h light cycle and constant humidity. Animals were killed by decapitation after administration of 90 mg/kg pentobarbital.

#### Experiments

Pregnant female Sprague-Dawley rats (gestation day 4) were purchased from Harlan Industries (Indianapolis, IN). They were housed individually in polycarbonate cages and allowed free access to water and pelleted food. They were randomly assigned to three groups and fed one of three semipurified diets made according to the AIN-93G diet formula (16), except that corn oil replaced soybean oil, and the protein source was casein (New Zealand Milk Products, Santa Rosa, CA). SPI (Protein Technologies International, St. Louis, MO) or WPH (New Zealand Milk Products, Santa Rosa, CA). Diets containing SPI had 430 mg total isoflavones/kg diet, including 276 mg/kg genistein and 132 mg/kg daidzein equivalents. L-Cysteine, L-methionine, L-phenylalanine, L-tryptophan and L-threonine were added to the SPI diet to the levels of the AIN-93G diet, as described previously (17). For the whey diet, 0.5 g/kg diet L-methionine and 2.1 g/kg diet L-phenylalanine were added to the AIN-93G diet levels. Female offspring were weaned to the same diet as their mothers. Starting at age 32 d, the stages of the estrous cycle were determined by daily vaginal smears. Rats exhibiting two consecutive 4- or 5-d cycles were used in the present study. Starting at age 47 d, females (n = 8–12) in metastases were orally gavaged with sesame seed oil or 65 mg/kg body wt DMBA (Sigma) dissolved in sesame oil. Rats were killed 24 h later and livers and the right abdominal no. 4 mammary gland were harvested as described by Russo et al. (13) for biochemical analysis.

#### Mono-oxygenase activities

Ethoxyresorufin-O-deethylase (EROD), a rat CYP1A1 selective activity (18), and methoxyresorufin-O-deethylase (MROD), an activity suggested to be selective for CYP1A2 (19), were measured by following the formation of resorufin spectrophotometrically at 536 nm (excitation) and 566 nm (emission) according to the method of Burke and Mayer (20) using an RF-5501PC scanning spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) under conditions of linearity for incubation time and protein.

#### Western immunoblot analysis

CYP1A, AhR and ARNT were measured in total mammary protein prepared using TRI Reagent (MRC, Cincinnati, OH) according to the manufacturer’s directions and the method of Varela and Ip (21). Hepatic CYP1 proteins were measured from microsomes. Hepatic AhR and ARNT were measured in total protein prepared by homogenizing 100 mg liver into ice-cold tissue lysis buffer [20 mmol/L Tris, pH 7.4; 0.15 mol/L NaCl; 1 mmol/L EDTA; 1 mmol/L EGTA; 1% Triton X-100; 2.5 mmol/L sodium pyrophosphate; 1 mmol/L β-glycerophosphate; 1 mmol/L Na3VO4; 1.0 mg/L leupeptin; and 1 mmol/L 4-2-aminoethylbenzenesulfonyl fluoride]. Proteins were resolved on 100 g/L polyacrylamide gels and transferred to a Hybond-P membrane (Amersham Pharmacia Biotech). Membranes were blocked at 8 h at room temperature with shaking in Tris-buffered saline plus Tween (TBST) [10 mmol/L Tris-buffered saline, 0.130 mol/L NaCl, 2.7 mmol/L KCl, pH 7.4; plus 100 g/L milk powder containing 50 μL milk powder containing 50 μL milk powder over night at 4°C with shaking. After washing three times in TBST, the membranes were incubated for 1 h at room temperature in TBST plus 50 g/L milk gel containing horseradish peroxidase–conjugated secondary IgG (1:5000–10000). Membranes were washed three times in TBST, and the proteins were visualized using the enhanced chemiluminescence plus system (ECL Plus; Amersham Pharmacia Biotech) and detected by autoradiography. Immunoprecipitation was obtained by densitometric scanning of the resulting autoradiographs using a Bio-Rad GS525 molecular imager (Richmond, CA).

#### Relative reverse transcriptase–polymerase chain reaction of CYP1A, AhR and ARNT mRNA levels

Total RNA was extracted using TRI Reagent (MRC) according to the manufacturer’s directions. First-strand cDNAs were synthesized from total RNA using the Reverse Transcription System (Promega, Madison, WI) according to the manufacturer’s protocol. CYP1A1, CYP1A2, CYP1B1 and β-acin primer sequences were described previously (22). The AhR (GenBank accession no. U04860) and ARNT (GenBank accession no. U61184) primer sequences described using Primer Designer software version 1.01 (Scientific & Educational Software, Durham, NC) were as follows: RAHR-F1, 5’-CAGAGAGCA TCAGAAATGAGGAG-3’ (AhR forward), and RAHR-R2, 5’-TGACCATTGGCT-GACTGTGAG TT-3’ (AhR reverse); RARNT-F1, 5’-CGTGTCGAAGTCGATCAAGA-3’ (ARNT forward), and RARNT-R1, 5’-CCGGAGAAAGACATGGCAGTTA-3’ (ARNT reverse). Polymerase chain reaction (PCR) was performed in a total volume of 25 μL of PCR buffer containing 1.1 mmol/L MgCl2, 0.2 mmol/L each primer, 1 unit of RedTaq DNA polymerase (Sigma) and 2 μL of
first-strand cDNA containing deoxynucleoside triphosphates. The amplification cycle number was determined to keep amplification of the desired products in the linear range to avoid the "plateau effect" associated with increased numbers of PCR cycles. The PCR cycle started at 95°C for 3 min followed by a three-step cycling: denaturation at 95°C for 30 s, annealing at 68°C (CYP1A1, CYP1A2, CYP1B1 and β-actin) or 58°C (AhR and ARNT) for 1 min and extension at 72°C for 1.5 min. Amplification cycle numbers were as follows: liver CYP1A1 and AhR, 25 cycles; liver CYP1A2 and β-actin, 23 cycles; liver CYP1B1, 27 cycles; liver ARNT, 34 cycles; mammary CYP1A1, CYP1B1, AhR and β-actin, 25 cycles; mammary CYP1A2, 38 cycles; and mammary ARNT, 37 cycles. This was followed by a final extension step at 72°C for 10 min. The PCR products were fractionated through 20 g/L agarose gel at 120 V and visualized by dual staining with ethidium bromide (Sigma) and cybergreen (Molecular Probes, Eugene, OR). The bands were analyzed by video densitometry; the areas of the peaks were calculated in arbitrary units. The relative value was generated by calculating the ratio of the arbitrary units of the CYP1, AhR or ARNT amplicon to that of β-actin.

**Statistical analysis.** Statistical analysis of DMBA effects in the three diet groups was performed by two-way ANOVA followed by the Tukey test using the SigmaStat Software (Jandel Scientific Software, San Rafael, CA) with P < 0.05 considered significant. Where just comparing diet effects (Table 3), one-way ANOVA followed by Student-Newman-Keuls multiple comparison was used. Data are presented as mean ± SEM.

**RESULTS**

**Induction of hepatic EROD and MROD activities.** Body weight, liver weight and yield of microsomal protein for rats fed the three different diets and treated with sesame oil vehicle or DMBA were measured, and there were no significant differences among groups (data not shown). DMBA induced the metabolism of EROD and MROD by eight- and threefold, respectively, in the CAS-fed rats (Table 1). The DMBA-induced activities of EROD and MROD were lower (P < 0.05) in the SPI-fed rats compared with the CAS-fed rats.

**Induction of liver and mammary gland CYP1A1, CYP1A2 and CYP1B1 levels.** The constitutive expression of hepatic CYP1A1 mRNA was lower (P < 0.05) in the WPH-fed rats than in the SPI- or CAS-fed rats (Figs. 1 and 2 and Table 2). However, this diet-dependent mRNA difference did not translate to differences in protein expression (Table 2). There was no constitutive CYP1B1 mRNA or protein detected in any diet group. The hepatic DMBA-induced mRNA expression for CYP1A1, CYP1A2 and CYP1B1 was lower (P < 0.05) in SPI- and WPH-fed rats compared with CAS-fed rats. At the protein level, there was a decrease (P < 0.05) in DMBA-induced expression for CYP1A1 in the SPI-fed rats compared with the CAS-fed rats with no differences in CYP1A2.

In mammary gland, there were no effects of diet on constitutive mRNA levels for CYP1A1, CYP1A2 or CYP1B1. The constitutive mammary gland CYP1A1, CYP1A2 and CYP1B1 protein expression was lower (P < 0.05) in the SPI-fed rats compared with the CAS-fed rats.

**TABLE 1**

<table>
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<td>648 ± 247</td>
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<tr>
<td></td>
<td>SPI</td>
<td>225 ± 45</td>
<td>685 ± 140b*</td>
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<tr>
<td></td>
<td>WPH</td>
<td>265 ± 85</td>
<td>662 ± 165ab*</td>
</tr>
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</table>

1 Values are means ± SEM, n = 8–12. a,b Diet group means within a treatment not sharing a superscript differ (P < 0.05). * Sesame oil and DMBA-induced means within a diet group differ (P < 0.05).
2 7,12-dimethylbenz-(a)anthracene; 65 mg/kg body wt.
3 EROD (Ethoxyresorufin-O-deethylase); MROD (methoxyresorufin-O-demethylase).
4 P = 0.055 (CAS vs. WPH).

**FIGURE 1** Representative Western immunoblots of CYP1A1, CYP1A2 and CYP1B1 protein expression in liver (A) and mammary gland (B) from casein (CAS)-, soy protein isolate (SPI)- or whey protein hydrolysate (WPH)-fed rats with or without DMBA treatment. Westerns are representative samples (n = 6–12/group) of hepatic microsomal protein (A) or mammary total protein (B) performed as described in Materials and Methods.

**FIGURE 2** Representative relative reverse transcriptase–polymerase chain reaction (PCR) analysis of CYP1A1, CYP1A2, CYP1B1 and β-actin steady-state mRNA levels in liver (A) and mammary gland (B) from casein (CAS)-, soy protein isolate (SPI)- or whey protein hydrolysate (WPH)-fed rats with or without DMBA treatment. PCR products were prepared and analyzed from samples (n = 6–12/group) as described in Materials and Methods.
common milk protein, WPH, had an effect on the incidence of mammary tumors compared with CAS-fed rats (5). Additionally, in the same study, we demonstrated that a diet made with SPI-fed rats had lower AhR and ARNT protein levels (. Table 3). The mechanisms by which these CYP1 proteins reported in Table 2. The mammary gland AhR expression in SPI- and WPH-fed rats compared with the CYP1A1 and CYP1B1 in the SPI- and WPH-fed rats compared with the CAS-fed rats.

**Diet-dependent effects on liver and mammary gland AhR and ARNT protein expression and mRNA steady-state levels.** Liver and mammary gland AhR and ARNT mRNA expressions were greater (P < 0.05) in the SPI-fed rats compared with the WPH- or CAS-fed rats (Figs. 3 and 4 and Table 3). Hepatic AhR and ARNT protein expressions were not different among diet groups. In the mammary gland, the SPI-fed rats had lower AhR and ARNT protein levels (P < 0.05) and these corresponded to the lower DMBA-induced CYP1 proteins reported in Table 2. The mammary gland AhR protein level in the WPH-fed rats was greater (P < 0.05) than in the CAS-fed rats, and this corresponded to the reduced expression of CYP1B1 (P < 0.05) mRNA and protein levels in the WPH-fed rats after DMBA treatment (Table 2).

**DISCUSSION**

Previous studies have reported that the incidence of either spontaneous mammary tumors (23) or chemically-induced mammary tumors are lower in rats fed diets made with soy flour or soy protein isolate (24–28). Similarly, we have recently reported that female Sprague-Dawley rats fed diets containing SPI had an ~25% lower incidence of DMBA-induced mammary tumors compared with CAS-fed rats (5). Additionally, in the same study, we demonstrated that a diet made with a common milk protein, WPH, had an ~45% reduced incidence of DMBA-induced tumors. The mechanisms by which these dietary proteins reduce mammary gland cancer are not known but could be related to the CYP1A1 and CYP1B1 phase I enzyme–dependent metabolism of DMBA. DMBA is a procarcinogen that is metabolized to its ultimate carcinogenic metabolite by CYP1A1 and CYP1B1 enzymes, and DMBA

**TABLE 2**

Effects of DMBA and diets made with casein (CAS), soy protein isolate (SPI) or whey protein hydrolysate (WPH) on hepatic and mammary gland CYP1 enzyme protein and mRNA steady-state expression in female rats

<table>
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<tr>
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<td>WPH</td>
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<td>ND</td>
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1. Absorbance density units (ADU) of the ratio of CYP1 to β-actin mRNAs; mean ± SEM (n = 8–12).
2. Absorbance density units (ADU); mean ± SEM (n = 6–12). a,b,c Diet group means not sharing a superscript differ (P < 0.05). * Sesame oil and DMBA-induced means within a diet group differ (P < 0.05).
3. 7,12-Dimethylbenz(a)anthracene; 65 mg/kg body wt.
4. Not detected (ND).

**FIGURE 3** Representative Western immunoblots of aryl hydrocarbon receptor (AhR) and AhR-nuclear translocator (ARNT) protein expression in liver (A) and mammary gland (B) from casein (CAS)-, soy protein isolate (SPI)- or whey protein hydrolysate (WPH)-fed rats. Westerns are of representative samples (n = 6–12/group) of hepatic or mammary total protein performed as described in Materials and Methods.
induces its own metabolism by upregulating expression of CYP1A1 and CYP1B1 enzymes (14,15). This potential mechanism is supported by our previously reported study that measured a reduction in poly cyclic aromatic hydrocarbon-induced hepatic CYP1A1 expression in male rats fed SPI (8).

In the current report, we measured the constitutive expression and DMBA-induced expression of CYP1A1, CYP1A2 and CYP1B1, because CYP1A1 and CYP1B1 bioactivate DMBA and all three enzymes share regulation through the AhR, thereby allowing insight into potential diet-dependent mechanisms. In female rats fed SPI or WPH diets, DMBA-induced mammary CYP1A1, CYP1A2 and CYP1B1 expressions were significantly lower than in CAS-fed rats. CYP1A1 possesses both EROD and MROD activities (18), and the decrease in hepatic DMBA-induced EROD and MROD activities may be the result of the reduced CYP1A1 protein expression alone, since CYP1A2 protein was not affected in the liver. However, in the mammary gland, concomitant decreases in CYP1A2 and CYP1A1 mRNA and protein were observed in DMBA-treated rats, suggesting a possible role for CYP1A2 in the mammary gland as well.

In addition to the CYP1A1 effects in the liver, DMBA-induced CYP1A1, CYP1A2 and CYP1B1 protein expression in the mammary gland and this induction was lower in SPI- and WPH-fed rats. Mammary gland CYP1 activities were not measured because of the limited tissue available; however, decreased activities for these enzymes would also be expected based on reduced protein expression. CYP1B1 catalyzes the formation of the highly mutagenic DMBA 3,4-dihydrodiol-1,2-epoxide metabolite thought to be responsible for mammary cancer initiation (15). Such a reduction in relative expression of these phase I enzymes may be the result of the reduced CYP1A1 protein expression alone, since CYP1A2 protein was not affected in the liver. However, in the mammary gland, concomitant decreases in CYP1A2 and CYP1A1 mRNA and protein were observed in DMBA-treated rats, suggesting a possible role for CYP1A2 in the mammary gland as well.

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1 Absorbance density units (ADU) of the ratio of CYP1 to β-actin mRNA; mean ± SEM (n = 8–12).
2 Absorbance density units (ADU); mean ± SEM (n = 6–12).

a,b,c Diet group means not sharing a superscript differ (P < 0.05).

AhR (aryl hydrocarbon receptor); ARNT (AhR-nuclear translocator).
mRNA levels but lower AhR and ARNT protein levels just before DMBA treatment in the mammary gland. Thus, SPI may be acting to inhibit the expression of AhR and ARNT proteins, which results in reduced CYP1 expression. It should also be noted that a similar trend occurred with hepatic AhR and ARNT expression in SPI-fed rats. However, WPH-fed rats had essentially the same ARNT protein levels but elevated AhR protein at the time of DMBA treatment, yet had significantly lower levels of CYP1 protein expressed in the mammary gland. Thus, these AhR and ARNT effects are important in CYP1 expression in the mammary gland, the mechanisms by which SPI and WPH act to reduce CYP1 expression differ substantially, whereby SPI works by inhibiting AhR and ARNT expression and WPH does not.

In the mammary gland, WPH caused a nearly 100% increase in constitutive AhR protein expression and this may account for the increased expression of CYP1B1 measured in WPH-fed rats. This is supported by in vitro molecular biology studies demonstrating that the AhR regulates both constitutive as well as inducible CYP1B1 expression (38). The mechanism for the increased AhR expression may result from the action of growth factors and/or growth factor–like peptides in WPH. Numerous studies have reported that whey contains several hormones and growth factors including insulin, bombesin, prolactin, insulin-like growth factor (IGF)-1, IGF-2, platelet-derived growth factor and fibroblast growth factor (39,40). Growth factors such as platelet-derived growth factor and basic fibroblast growth factor have been reported to induce AhR expression (41). Alternatively, these WPH growth factors and/or peptides derived from whey hydrolysis may coregulate the decreased DMBA induction of CYP1 with no direct effect on the AhR or ARNT levels, but with an effect on the expression or activity of transcriptional coactivators or other transcription factors that regulate the CYP1 genes. The WPH used in this study contains peptides that are hydrolysate products from the bovine milk protein and from milk-derived growth factors. In addition, further hydrolysis of protein and peptide products by the gastrointestinal tract will likely yield other peptides. We have hypothesized that some of the proposed health benefits of whey protein are due to the actions of these peptides. For example, in preliminary studies, we have determined that fractionated peptides alter the cell signals that lead to changes in proliferation (42).

A potential mechanism for these soy effects might be attributed to the soy-associated isoflavones, such as genistein, that have been characterized as estrogens (phytoestrogens) because of their affinity for estrogen receptor (ER)-α and ERβ (43,44). Soy phytoestrogens may play a role in the CYP1 effects measured here since there is evidence that estrogens can inhibit the induction of CYP1A1 and CYP1B1 (45–48). Estrogens may downregulate the protein expression of AhR and/or ARNT, thereby reducing AhR-induced gene transcription. Evidence for this has been reported for ARNT where decreased mRNA expression was lowest at estrous during the cycle in female rats, suggesting that hormones such as estrogen may downregulate ARNT expression (49). Alternatively, both the ER and AhR interact with the same nuclear coactivators during transcriptional regulation (50–53). A possible mechanism for ER-AhR “crosstalk” and antagonism may involve ER binding all of the available nuclear coactivators needed for transcriptional regulation by the AhR. Thus, both in vivo and in vitro studies suggest that estrogen can antagonize DMBA induction of CYP1A1 and CYP1B1, and this may occur by several mechanisms.

In summary, the results reported here demonstrate that DMBA inducibility of CYP1 is dependent on the source of dietary proteins. Factors found in SPI and WPH possibly interact with the AhR-ARNT pathway leading to decreased induction of the CYP1 protein. Rats fed SPI diets had lower DMBA-induced CYP1A1 hepatic activity compared with those fed WPH or CAS. In addition, there were decreases in all three mammary gland CYP1s in DMBA-induced SPI-fed rats compared with the CAS-fed rats. Because there were larger decreases in DMBA-induced mammary gland tumors in WPH-fed rats compared with SPI- or CAS-fed rats (5), the decreases in CYP1s in the SPI-fed rats appear to only partly contribute to the reduction in tumors measured. Additional cancer prevention may be derived from diet-induced increases in mammary gland differentiation. We have measured increased mammary gland differentiation in WPH-fed rats compared with both SPI- and CAS-fed rats (54). Thus, in the WPH-fed rats, the combined decreases in mammary DMBA-induced CYP1 expression, coupled with increased differentiation, may render the mammary gland in these rats more resistant to DMBA-induced mammary tumors, as compared with the SPI- or CAS-fed rats. The decreased mammary tumors in the SPI-fed rats relative to CAS-fed rats may be solely due to SPI-dependent inhibition of the CYP1 genes. The effects of WPH and SPI on mammary gland differentiation and CYP1 expression are currently under investigation.

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LITERATURE CITED


