

# Fourth International Symposium on the Role of Soy in Preventing and Treating Chronic Disease

## The Health Consequences of Early Soy Consumption<sup>1,2</sup>

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**ABSTRACT** Infants fed soy formula are the segment of the U. S. population that consumes the most soy. Before birth and after weaning, most Americans are not exposed to appreciable levels of soyfoods other than foods that have small amounts of processed soy components. The opposite scenario occurs in Asia, because Asians are more likely to consume relatively high levels of soyfoods throughout life, except between birth and weaning, when breastfeeding or milk-based formula are common. Soy formula is made with soy protein isolate containing isoflavones (SPI+) and supports normal growth and development in term infants. Recent data suggest that there are no long-term adverse effects of early exposure to soy formula through young adulthood. It is as yet unknown whether soy formula consumption by infants will result in health problems or benefits upon aging, but multigenerational animal studies with diets made with SPI+ have not revealed any problems. Soy isoflavones can function as estrogen agonists, antagonists or selective estrogen receptor modulators, depending on the conditions, and much research has focused on health effects of purified isoflavones. Results from several studies suggest that the effects of diets made with SPI+ differ significantly from those of diets to which purified soy isoflavones are added. Furthermore, it seems that soy protein processed to contain lower levels of isoflavones also provides significant health benefits. Further research is needed to confirm the results of the few studies that have been conducted and new studies are needed to investigate the more subtle effects that could occur during development or that could surface later in life. J. Nutr. 132: 559S–565S, 2002.

**KEY WORDS:** • soy • genistein • development • infant formula • disease prevention

In 1929 Hill and Stuart (1) reported the use of soy formula for infants with allergies to cow's milk protein. Since then, soy formula has been used for several other related medical indications including postdiarrhea lactose intolerance, galactosemia and primary lactase deficiency. Early soy formula had several deficiencies, and infant acceptability and growth were not equal to those for milk-based formula. However, over the years, the quality of soy formula has improved, in good part because the U. S. Food and Drug Administration established

safety and quality standards for infant formulas. Although the American Academy of Pediatrics recommends breastfeeding over formula feeding, it also recommends isolated soy protein-based formulas as a safe and effective alternative for providing appropriate nutrition for normal growth and development for term infants whose nutritional needs are not being met from maternal breast milk or cow's milk-based formulas (2). For mothers who are not able or willing to breastfeed, the American Academy of Pediatrics recommends cow's milk-based formula over soy formula, presumably because of the lack of demonstrated advantages of soy-based formula coupled with its much shorter safety history. It is important to note that no health advantages are documented for feeding cow's milk formulas over soy-based formulas. There are, of course, other reasons why soy formula is fed, including the desire of parents to maintain a vegetarian lifestyle and increasing belief that soyfoods provide the potential benefits of dietary factors in preventing chronic diseases. Marketing data and hospital discharge records suggest that ~25% of the nearly 4 million newborns in the United States consume soy infant formula (2).

The compositions of breast milk, cow's milk formula and soy formula differ substantially. Because the amino acid composition, vitamins, minerals, protein level, lipid level and total calories are all adjusted in formulas to provide the needs for growth and development, the major differences in these three

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TABLE 1

Plasma concentrations of 17 $\beta$ -estradiol (E<sub>2</sub>) and total isoflavones in women and infants

	Approximate plasma concentrations	
	E <sub>2</sub> nmol/L <sup>1,2</sup>	Total isoflavone nmol/L <sup>1</sup>
Maternal	148	744 <sup>3</sup>
Cord blood	148	831 <sup>3</sup>
Amniotic fluid		779 <sup>3</sup>
Neonate		
Milk formula	0.00002	25 <sup>2</sup>
Soy formula		7000 <sup>2</sup>
Breast milk		25 <sup>2</sup>
Follicular phase	0.3	
Midcycle phase	2.2	
Luteal phase	0.7	
Menopause	0.07	

1 Concentrations at higher end of reported range.

2 Values estimated from Setchell et al. (3).

3 Japanese women only (6), American women would typically be <10 nmol/L.

infant diets are the sources of protein, lipids and carbohydrates. Commercially available soy formula marketed in the United States is made with soy protein isolate (SPI+).<sup>4</sup> SPI+ has several phytochemicals associated with it, including isoflavones, which are present as glucosides in the protein matrix, but circulate in the body mainly as glucuronide and sulfate conjugates, and a minor amount of the aglycone. As discussed later, soy isoflavone aglycones are often referred to as phytoestrogens and, thus, are frequently compared with 17 $\beta$ -estradiol (E<sub>2</sub>). Although some of the soy phytochemicals found in soy formula are also present in human or cow's milk, their concentrations are low (3–5).

#### Plasma soy isoflavone concentrations

Because of obvious ethical and safety reasons, data on the fetal plasma or tissue isoflavone concentrations are essentially nonexistent, and the data on infant plasma concentrations are limited. However, two studies provide insights into the likely isoflavone exposure throughout the life cycle. Adlercreutz et al. (6) demonstrated that there are significant concentrations of isoflavonoids transported from the maternal to fetal compartment of Japanese women who consumed their usual diet rich in isoflavone sources, including soyfoods, and Setchell et al. (3) reported the plasma isoflavone concentrations of infants fed breast milk, cow's milk formula or soy formula as well as the approximate plasma E<sub>2</sub> concentrations of women and infants. The numbers of subjects were limited in both studies and only the higher values of the plasma ranges are summarized in Table 1 to illustrate that isoflavone phytoestrogens can attain very high concentrations early in human development. The mean total maternal plasma isoflavonoid concentration of Japanese women who consumed their normal diets was 233 nmol/L (range: 19–744) and the mean concentrations of cord blood and amniotic fluid at birth were 299 nmol/L (range: 58–831) and 223 nmol/L (range: 52–779), respectively (6). Thus, high levels of isoflavonoid phytoestrogens are trans-

ferred from the maternal to the fetal compartment. These concentrations are probably an underestimate of the peak values because these women did not eat during their labor and the blood sampling was taken at the hospital during delivery. This suggests the reported isoflavone concentrations are likely to be lower than the maximal concentrations attained throughout the day. Infants are fed every few hours, and given that the half-life of the isoflavones is longer in the period between feedings, the values reported by Setchell et al. (3) for plasma isoflavones are probably close to the maximal concentrations (Table 1). Therefore, accounting for the timing of sample collection in relationship to meals and the differences in methodology between laboratories, the isoflavone concentrations of infants fed soy formula and the fetuses of Asian women are most likely within the same general concentration range (i.e., a low  $\mu$ mol/L range). Although the estrogenic potency of isoflavones is estimated to be lower than that of E<sub>2</sub>, the plasma isoflavone concentrations are ~50–100 times higher in infants fed soy formula than the E<sub>2</sub> levels achieved by women during pregnancy and nearly 3000 times higher than the E<sub>2</sub> concentrations achieved during the estrogen surge of the menstrual cycle.

#### Soy exposure in Asian and American infants

The dietary habits of Americans, especially with respect to soyfoods, differ substantially from those of Asians. Figure 1 is a cartoon of hypothetical circumstances using the maternal and infant plasma isoflavone concentrations of the studies by Setchell et al. and Adlercreutz et al. (3,6) to illustrate the differences in exposure to isoflavonoids between Asians and Americans from conception through weaning. In this diagram, the Asian fetus is exposed to the same high isoflavone levels found in the maternal circulation during pregnancy, based on the cord blood and amniotic fluid isoflavone data (6). Asian infants consume either milk formula or breast milk for the first few months of life, with other foods (such as fermented soy paste) often being introduced early and continuing through completion of the weaning process, which ends at ~14 mo old in the diagram. Thus, their plasma isoflavone levels would be expected to decline at birth, because most infants are breastfed

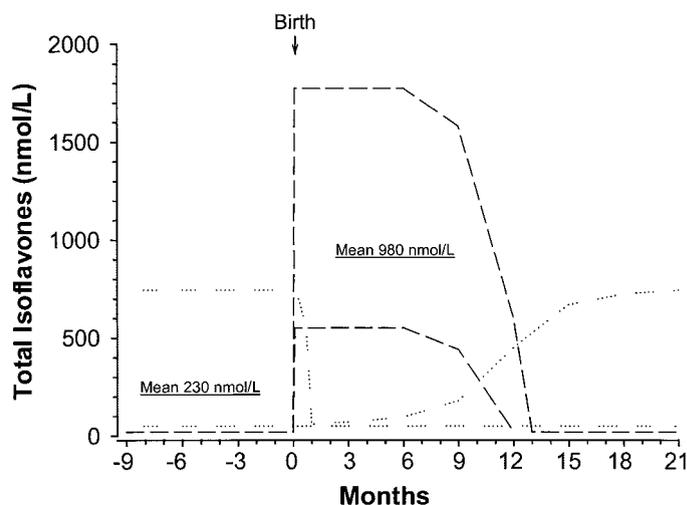


FIGURE 1 Hypothetical serum concentrations profile of isoflavones from conception through weaning in typical Asians and Americans. The values represent the range of isoflavonoids reported by Adlercreutz et al. (6) for Japanese (dotted lines) or reported by Setchell et al. (3) for Americans fed soy infant formula (dashed line).

<sup>4</sup> Abbreviations used: AhR, aryl hydrocarbon receptor; ARNT, AhR-nuclear translocator; DMBA, dimethylbenz(a)anthracene; E<sub>2</sub>, 17 $\beta$ -estradiol; SPI+, soy protein isolate; SPI-, soy protein isolate without isoflavones.

or fed milk-based formula. These levels would remain low until soyfoods (or other isoflavone-containing foods) are introduced and would increase to the maternal levels if the same dietary habits are acquired by the child. In the United States, most pregnant women do not consume significant isoflavones, fetal exposure is low, and 75% of American infants will not be exposed to diets high in soy and their plasma isoflavone levels remain low. However, American infants fed soy formula would be expected to have high plasma levels starting immediately after birth and until they are weaned, at which time the plasma levels would drop to  $< 20$  nmol/L and probably remain low thereafter. The point of the cartoon is that there are substantial differences between Asian and American exposure to soyfoods. The health implications of this exposure profile have not been fully explored.

### *The debate over early soy exposure*

Soyfoods are known to provide health benefits, and because the soy isoflavones are active in many cellular systems (7–10), much attention has been focused on their possible role in health. Although the isoflavones have effects that are estrogen receptor-mediated and nonestrogen receptor-mediated, the former has captured the most attention of both scientists and the lay public. Soy isoflavones, often referred to as phytoestrogens, bind to estrogen receptors and function as estrogen agonists, antagonists or selective estrogen receptor modulators, depending on the tissue, cell type, isoflavone concentration and other conditions, such as hormonal status, age, etc. (11–15). The high isoflavone concentrations of blood (and presumably tissue) of infants fed soy formula are of interest because humans do not normally have high concentrations of estrogens until after puberty. This raises the question of short- and long-term health effects of such hormonal actions on the development of infants. Genistein has been reported to be 3 to 400,000 times less potent than E2 (12,13,16,17), with a value of 1:1000 frequently used. However, potency has been studied mainly in *in vitro* systems or in animals and the true potency in target cells of human infants has never been reported. Thus, given the uncertainty about the potency of isoflavones in infants and the high plasma isoflavone concentrations during postnatal life when estrogenic factors are usually very low, a scientific debate has developed and centers on the idea that isoflavones in soy formula may have adverse effects (18–22).

Central to the debate on the safety of soy formulas are reports of adverse health effects of isoflavones on animal reproductive systems. Perhaps the most well-known complication of isoflavones is the reproductive disorders or infertility in Australian sheep that graze on clover with a high isoflavone content, often referred to as clover disease (23,24). This clover disease was caused by extremely high concentrations of equol, a potent estrogenic isoflavone, on the mature reproductive system of seasonal breeding ruminant animals at a critical endocrinological period when disruption of complex and usually well-orchestrated hormonal events caused infertility. However, clover disease is not applicable to infants consuming soy formula because equol concentrations are nearly undetectable in infants consuming soy formula, the mature reproductive system disrupted by equol in clover disease is not present in infants and will not be until after puberty, and several other physiological and development systems of adult sheep and human infants are not comparable.

No human data support the toxicity of soyfoods as marketed in the United States, especially regarding reproductive competency. In countries in which soy has been consumed at the greatest daily intake for centuries, the population has in-

creased at rates often exceeding the national ability to support usual population needs, such as food supplies, health care, etc. Women who consume soyfoods that result in the high circulating concentrations of isoflavones are capable of conceiving, taking the pregnancy to term, delivering normal infants, normally lactating, and otherwise caring for their infants. Because these women consumed soyfoods before pregnancy and continued eating soy during pregnancy and during lactation, soyfoods do not seem to have adverse effects on early human development or later reproductive performance. No reported epidemiological evidence suggests that soyfoods have adverse effects at these important critical periods during which other hormones and drugs, such as diethylstilbestrol or alcohol, were reported to have damaging developmental and health effects. For example, to our knowledge there have been no reports of soyfood-associated adverse health effects of Japanese newborns, suggesting that perinatal exposure to the high isoflavone concentrations achieved in utero (6) or by soy infant formula (3) are not likely to result in adverse health effects, especially not the type of abnormalities ascribed to diethylstilbestrol or fetal alcohol syndrome. Furthermore, multiple generations of people have consumed soyfoods without adverse effects of early exposure to soy when these are consumed in the context of a normal diet. Infant formulas have been carefully studied, are regulated by the Food and Drug Administration, and are monitored by advocate groups most attuned to children's welfare, such as the American Academy of Pediatrics, for effects on growth, development, safety, and general health. It should also be pointed out, however, that large studies focusing on these issues have not been conducted.

Soyfoods have a long history in Asia of being safe, and the evidence continues to mount on the health benefits of soy. No similar human database exists on the health effects of purified isoflavone aglycones. Many animal studies have been conducted on the effects of isoflavones given by injection or oral gavage or diets made with purified soy isoflavones. Although it is important to study the health effects of high-dose purified isoflavones in adults, especially given the appearance of purified genistein and mixed soy isoflavones now marketed across the United States, it is not clear what bearing these results have on the health effects of infants consuming soy formula, where the high levels of isoflavones are accompanied by several other soy components, including proteins, peptides, a mixture of isoflavones and saponins. Currently, there are no approved infant formulas to which purified isoflavones are added, nor are there ever likely to be any. There is no evidence that the effects of purified isoflavones are the same as either SPI+ or soy infant formula. In fact, results from several studies described below suggest that SPI+ effects differ substantially from the effects of purified isoflavones.

Millions of American infants have been fed soy formula over the past three decades. Several studies have demonstrated that soy formula supports normal growth and development in term infants (25–29). When growth was studied over the 1st y of life, body weight gains and body length of infants were virtually the same whether the infants were fed soy formula or cow's milk-based formula or breastfed (30). Recently, Strom et al. (31) reported on 811 young adults who were fed cow's milk-based formula or soy formula as infants; men and women between 20 and 34 y old were studied to determine the long-term health consequences of early soy intake. There were a few statistically significant differences between the cow's milk formula and soy formula groups, but no differences were found in growth, development, puberty, reproductive function, pregnancy outcomes or a host of other variables. However, the number of subjects was low and does not allow the

determination of whether some of the pregnancy outcomes (slightly higher incidences of preterm or stillborn deliveries and multiple births) are biologically meaningful. Furthermore, the subjects were too young to determine the risk of developing most chronic diseases that occur later in life, and the population selected for study was limited to mostly white, well-educated Midwestern Americans and may not be applicable to a wider population. Nonetheless, these data add to the already large database suggesting that soy formula is safe and effective in promoting normal growth and development of term infants.

One issue related to soy infant formula is the long-term health consequences of early consumption of these formulas. We have studied the effects of feeding the same SPI used in infant formulas to several generations of rats with the idea of establishing a situation similar to Asians who have high levels of soy intake throughout their lives. We fed AIN-93G diets (made with SPI) throughout their lives and found that male and female rats have the same breeding efficiency as rats fed commercial diets or AIN-93G diets made with casein (32). The numbers of offspring, gender ratios, birth weights, birth lengths, health and general appearance of soy-fed rats were the same as casein-fed rats. Indices of estrogenicity, such as weights of secondary sex organs, plasma estrogen concentrations and mammary gland development, were found to be normal. The only major effect was vaginal opening being 1 d earlier in soy-fed rats; the practical consequence of this finding is unclear, because earlier puberty has not been a recognized issue in Asia. However, this latter point has not been well-studied.

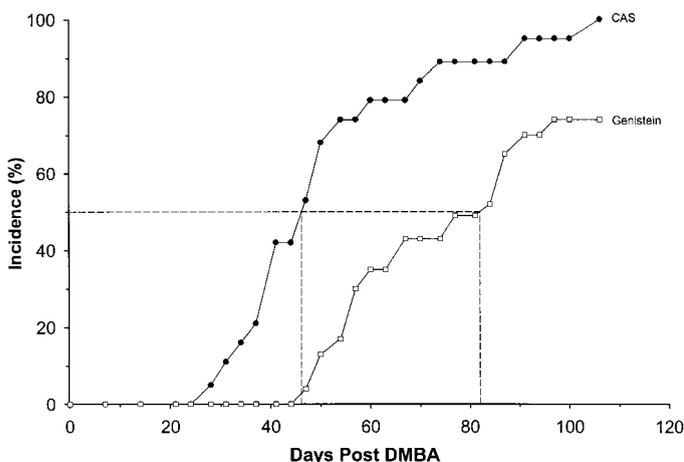
### Soy and cancer

Soy may have a role in the prevention of cancer, and even more important is the idea that early soy intake could prevent cancer that normally develops later in life, such as breast, colon or prostate cancer. In this regard, injections of high-dose genistein in newborn rats delayed the onset, reduced the incidence, and lowered the multiplicity of dimethylbenz(a)anthracene (DMBA)-induced mammary tumors (32–34) (Fig. 2). Reduced multiplicity of DMBA-induced mammary tumors

was also reported when genistein was added to the diet (35). There are four key features of these studies involving genistein: 1) these *in vivo* data provide direct evidence suggesting that at least some of the lower cancer incidence of Asians could be due to soy; 2) tumor initiation, and perhaps tumor promotion, were inhibited; 3) tumor onset was delayed; and 4) rats in these studies were exposed to genistein only in a limited segment of the early life and yet were apparently protected from cancer initiation later in life. This latter point is of extreme importance to possible health benefits of soy infant formula. In the studies reported in Figure 2, rats were treated on only 3 d of the first 6 d of life, and they were never exposed to soy or any soy component for the duration of the experiment (32). This suggests that the effects were essentially permanent in some rats, implying that infant rats can be exposed once to a soy component and be protected thereafter against chemically induced cancers. These data also support the notion that biochemical events occurring in a discrete period of early life can have long-lasting effects that could ultimately delay or prevent chronic diseases that normally occur later in life. This is important in light of the discussion of information in Figure 1, showing that American children are also exposed to SPI+ and its phytochemical factors for a defined period in early life (usually starting within days of birth). We are interested in determining whether soy formula can influence the health of infants or have long-lasting health effects.

Our research interests are in soyfoods (not purified isoflavones), especially soy formula and specifically the SPI+ that comprises the total protein source of these formulas. SPI+ is the soy protein currently used in infant formula, and although the isoflavone content varies somewhat between processing lots, our SPI+ diets contain total isoflavones at ~877 mg/kg. We have found that rats fed AIN-93G diets made with SPI+ have lower DMBA-induced mammary tumor incidence and multiplicity (32,36) as well as reduced incidence of azoxymethane-induced colon cancer (37). The mechanisms by which genistein and SPI+ reduce experimental mammary cancers have been investigated. Lamartiniere and coworkers (35,38,39) reported that neonatal genistein treatment increases rat mammary differentiation, resulting in decreased terminal end buds and increased lobules. Because mammary cancers develop in the epithelial cells of the terminal end buds, this would reduce the numbers of target cells available for mutations, which is consistent with the reduced numbers of DMBA-induced mammary tumors in rats either fed or injected with genistein. Thus, these studies suggest that the mechanism by which genistein protects against cancers in the Lamartiniere model is enhanced mammary gland differentiation, leading to a more mature gland with fewer target cells and less risk of developing cancer.

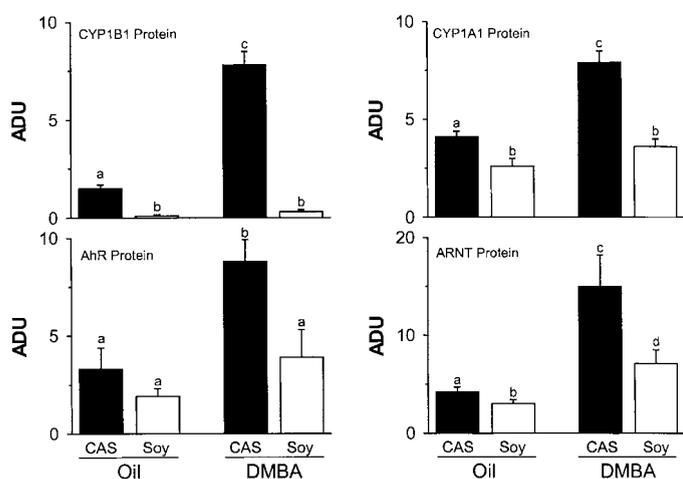
In our studies, female rats were fed AIN-93G diets made with SPI+ and the mammary glands were studied at 50 d old. Postnatal d 50 was selected for study because this is the age at which rats are treated with the procarcinogen, DMBA. Because initiation of mutagenesis by the highly mutagenic 3,4-dihydrodiol-1,2-epoxide metabolite of DMBA occurs at this time, the differentiation status will in part determine the risk of tumorigenesis. Therefore, from a target cell standpoint, rats fed AIN-93G diets made with SPI+ should have the same risk of developing mammary gland cancer as rats fed the same diet made with casein, but they do not. In these rats the gross mammary gland morphology (differentiation stage), epithelial cell apoptotic index, expression of estrogen and progesterone receptors, and mammary density were the same as for control rats fed the AIN-93G diets made with casein as the protein



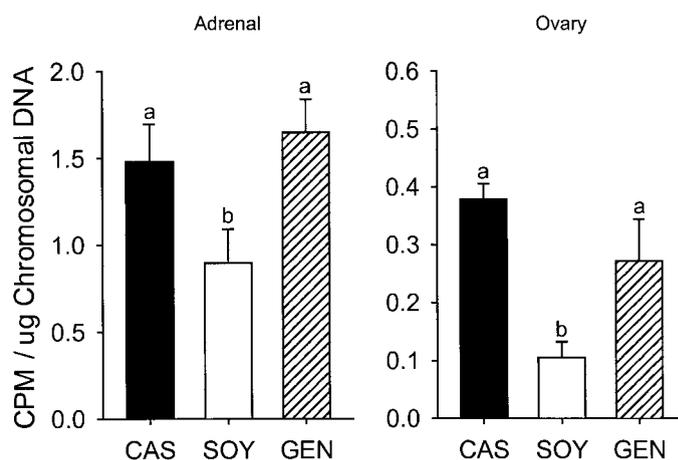
**FIGURE 2** Rats were injected with genistein at 500  $\mu\text{g/g}$  body weight at 2, 4 and 6 d of life and orally gavaged with dimethylbenz(a)anthracene (DMBA) at 80 mg/kg at 50 d old. Tumor incidence was determined by palpation. Rats were fed AIN-93G diets made with either casein (CAS) or the same diet to which 250 mg genistein/kg was added. Adapted with permission from Badger et al. (32).

source (40). That is, diets containing SPI+ do not reduce the number of terminal end buds by 50 d old. This represents a significant difference between feeding a diet with SPI+ and a diet to which purified genistein is added. Because rats were studied at exactly the same age, the stage of the estrous cycle was not controlled for; thus, to determine whether the stage of the estrous cycle could have any bearing on the results, we examined the mammary glands at the same day of the estrous cycle. We found essentially the same effects except for progesterone receptor expression, as determined by Western immunoblot analysis. In these rats there was 24% greater progesterone receptor expression in the terminal end buds but no differences in lobule progesterone receptor expression (41). These results are important because they suggest that the mechanisms by which SPI+ and purified genistein protect against chemically induced cancer seems to be completely different, or at least the SPI+ does not reduce mammary gland tumor incidence by increasing mammary gland differentiation as reported with genistein (35,38,39). Because the differentiation status of the mammary gland is such an important distinction between rats treated with SPI+ and genistein, further and more complete investigations are currently underway in our laboratory.

Because mammary differentiation did not seem to be significantly affected in rats fed AIN-93G diets with SPI+, we studied other possible mechanisms by which SPI+ could reduce the incidence of mammary gland tumors. We first studied the effects of phase I metabolism, because DMBA is a procarcinogen that must be metabolized to a carcinogen (activation stage) by cytochrome P450 enzymes, primarily those in gene family 1 (CYP1). One possible mechanism by which soyfoods could protect against toxic compounds such as procarcinogens would be to prevent activation by inhibiting enzymes necessary for the conversion from a procarcinogen to a carcinogen. This in turn would be expected to reduce the numbers of adducts and subsequent mutations that lead to mammary cancer. We demonstrated that rats fed SPI+ diets have reduced levels of hepatic CYP1A1 and mammary gland CYP1B1 and



**FIGURE 3** Rats were fed AIN-93G diets made with either casein (CAS) or soy protein isolate (Soy) from conception to adulthood. At 48–50 d old, rats were given DMBA at 65 mg/kg by oral gavage and were killed 24 h later. Western immunoblot analysis was used as described previously (35) to determine the level of CYP1A1, CYP1B1, aryl hydrocarbon receptor (AhR), and AhR-nuclear translocator (ARNT) proteins in the mammary gland. ADU = absorbance densitometric units. Values are means ( $\pm$  SEM), and values with different superscripts differ at  $P < 0.05$ .



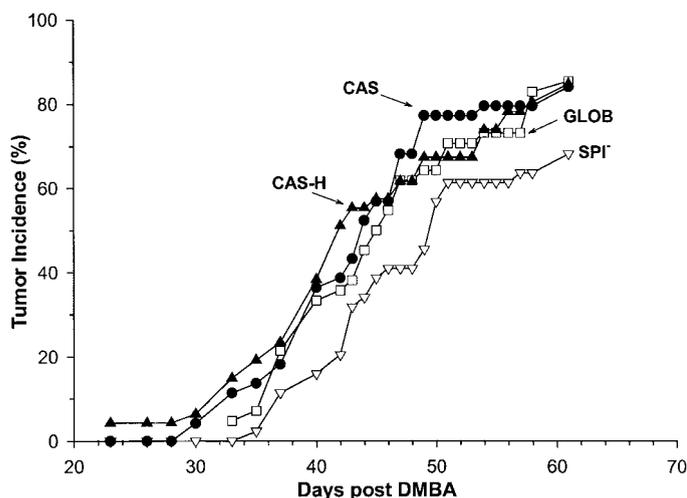
**FIGURE 4** Rats were fed AIN-93G diets made with either casein (CAS) or soy protein isolate (SOY) or CAS + 250 mg/kg genistein (GEN) from conception to adulthood. At 50 d old, rats were given [3H]DMBA at 65 mg/kg by oral gavage and were killed 24 h later. [3H]DMBA-DNA adducts were studied using the procedures of Upahyaya and El-Bayoumy (48). Values are means ( $\pm$  SEM), and values with different superscripts differ at  $P < 0.05$ . CPM = counts per minute.

CYP1A1 at the time of DMBA treatment (42) (Fig. 3). The expression of CYP1A1 and CYP1B1 is regulated by the DMBA-activated aryl hydrocarbon receptor (AhR). The AhR binds to a ligand and interacts with the AhR-nuclear translocator (ARNT) to form a nuclear AhR-ARNT heterodimeric complex that acts as a ligand-activated transcription factor that in turn binds to the xenobiotic response elements in the regulatory region of CYP1 genes (43). We studied transcription factor expression at the time of DMBA treatment and found lower AhR and ARNT protein levels in the cytosol and nucleus, respectively (Fig. 3). The downstream consequence of reduced DMBA activation would be lower target tissue carcinogen concentrations and fewer DNA adducts.

We further investigated the numbers of DMBA-DNA adducts in the ovary and adrenal glands (Fig. 4), tissues where CYP1B1 levels are reported to be extremely high relative to other organs (44–47). As predicted, we found reduced ( $P < 0.05$ ) ovarian and adrenal DMBA adducts in SPI+ -fed rats. Our data compliment those of Upahyaya and El-Bayoumy (48), who reported reduced DMBA-DNA adducts in the mammary gland of rats fed SPI-containing diets. Taken together, these data suggest that SPI+ is working to reduce the incidence of DMBA-induced mammary gland cancer by down-regulating CYP1A1 and CYP1B1, reducing DMBA-DNA adducts and cancer incidence (42). Thus, the mechanisms by which SPI+ (the same soy protein used in infant formula) reduces the experimentally induced mammary gland cancer seems to differ substantially from those of purified genistein as reported by Lamartiniere and coworkers (35,38,39).

#### Are purified soy isoflavones equivalent to spi+?

The apparent differences between the effects of soy protein and those of the purified soy isoflavone genistein are important enough to elaborate upon. There are now data in several areas that suggest that consuming SPI+ results in different biological effects than does consuming genistein without soy protein. There are five examples to discuss here. The first was discussed previously and is illustrated by the differences in mammary gland differentiation observed between studies from our laboratory in which rats were fed AIN-93G diets made with SPI+



**FIGURE 5** From conception to adulthood, rats were fed AIN-93G diets made with casein (CAS), soy protein isolate without isoflavones (SPI<sup>-</sup>), or hydrolyzed casein (CAS-H). Another group was fed a commercially available low-soy-protein diet (Harlan Global, GLOB). At 50 d old, rats were given DMBA at 65 mg/kg by oral gavage, and the mammary gland tumor incidence was followed.

(37,38) and the results from the Lamartiniere laboratory (35,38,39) in which NIH-76A diets were made with casein to which purified genistein was added. The differences between SPI<sup>+</sup> and genistein effects are dramatic, and there may be benefits from both SPI<sup>+</sup> and genistein in cancer prevention but by different mechanisms, as is discussed in the second example below.

The second example is the recent finding of Constantinou et al. (49) who fed rats a diet made with SPI<sup>+</sup> that had been extracted to remove the isoflavones (SPI<sup>-</sup>). They found significantly reduced DMBA-induced mammary gland tumors in rats fed SPI<sup>-</sup> diets compared with casein diets. We too have conducted such an experiment with similar results (Fig. 5). We compared the AIN-93G diet made with SPI<sup>-</sup> to other low-isoflavone diets and found that the SPI<sup>-</sup> diet reduced DMBA-induced mammary tumor incidence and multiplicity ( $P < 0.05$ ) and increased the median tumor latency ( $P < 0.05$ ) compared with the other diets (50). The total isoflavone content of our SPI<sup>-</sup> diet was 41 mg/kg diet, compared with 877 mg/kg in the SPI<sup>+</sup> diet. These results suggest that the cancer-preventing effects of soy do not involve high doses of isoflavones and that the protein (or more probably a peptide or protein fragment) or other nonisoflavone phytochemicals that may remain bound to the SPI<sup>-</sup> may have significant biological activity.

The third example relates to the cholesterol-lowering effects of soy protein. Studies in both animals and humans have demonstrated that SPI<sup>+</sup> intake reduces serum cholesterol (51). However, the most recent data demonstrate that whereas the maximal cholesterol-lowering effects are produced by feeding monkeys diets made with the SPI<sup>+</sup>, near maximal cholesterol-lowering effects (> 70%) are attained with SPI<sup>-</sup> processed to contain extremely low levels of isoflavones (52). Thus, this is a situation similar to the reduction of chemically induced mammary cancers described above with SPI<sup>-</sup> and again suggests that the high isoflavone concentrations are not required for at least some major potential health benefits of soy.

The fourth example is illustrated in Figure 4 with the DMBA-DNA adducts. In this experiment, rats were fed AIN-

93G diets made with casein, SPI<sup>+</sup>, or casein plus genistein. The two latter diets contained the same level of genistein equivalents (250 mg/kg). Rats fed the SPI<sup>+</sup> diet had significantly lower levels of DMBA-DNA adducts than did rats fed casein diets ( $P < 0.05$ ), but rats fed diets containing purified genistein did not, again suggesting that genistein and SPI<sup>+</sup> have different effects.

The fifth example relates to metabolism of bioavailable soy isoflavones. Data from recent reports of serum and brain isoflavone profiles of male rats fed diets with purified genistein, but without soy protein, differ from the profile in our male rats fed diets made with SPI<sup>+</sup>, although the total isoflavone intake was roughly the same. In those reports, genistein glucuronide equaled the total genistein concentrations in the serum (53), suggesting that essentially all the genistein is in the glucuronide form, and 100% of genistein in the brain was reported to be in the aglycone form (54). However, genistein metabolites differed substantially in our rats fed AIN-93G diets made with SPI<sup>+</sup>, with the percentages of genistein aglycone, genistein glucuronide, and genistein sulfate being 0.6%, 57.3% and 42.1% in serum and 36.4%, 34.1% and 29.5% in brain, respectively (55). These data suggest that genistein is metabolized differently when consumed as the aglycone or as a component of SPI<sup>+</sup>, perhaps because of the isoflavone mixture present in SPI<sup>+</sup> diets. Because methodological differences between laboratories could be a factor in these results, genistein metabolism in animals fed purified genistein or SPI<sup>+</sup> should be conducted in the same laboratory.

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