Fetal programming of colon cancer in adult rats: correlations with altered neonatal growth trajectory, circulating IGF-I and IGF binding proteins, and testosterone

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Abstract

We examined effects of dietary soy protein isolate (SPI) or genistein (GEN; soy isoflavone) during pregnancy on development of colon cancer in male progeny Sprague–Dawley rats. Four groups of rats were used: a lifetime casein-fed group (CAS; control diet), a lifetime SPI-fed group (positive control for protective effect of diet on colon carcinogenesis), a group whose dams received SPI only during pregnancy and CAS thereafter (SPI/CAS), and a group whose dams received CAS+GEN only during pregnancy and CAS thereafter (GEN/CAS). At 47 and 55 days of age, male progeny were administered the intestinal carcinogen azoxymethane (AOM). Tumors, endocrine status, and colon gene expression were evaluated at 20 week post-AOM. The SPI group had 47% decreased colon tumor incidence compared with the CAS group (P<0.05), whereas SPI/CAS, GEN/CAS, and CAS groups did not differ in this regard. Maternal-only SPI increased the percentage of animals bearing multiple colon tumors (P<0.05), an effect not mimicked by GEN. Serum insulin and leptin concentrations were decreased by lifetime SPI (P<0.05), whereas serum IGF-I was elevated in the SPI/CAS group (P<0.05). The SPI/CAS group had reduced serum testosterone levels (P<0.05) and exhibited a tendency for increased mucosal expression of IGF-I receptor and glucose transporter-1 mRNAs. Results indicate an effect of dietary protein type during pregnancy on colon tumor multiplicity and colon tissue gene expression, and serum IGF-I and testosterone in progeny rats as later adults.


Introduction

Diet is an important factor in the prevention as well as etiology of human cancers, including those of the gastrointestinal tract (Aggarwal & Shishodia 2006). Soy foods and soy constituents have received considerable attention for potential in reducing cancer risk (Badger et al. 2005). For example, epidemiological studies generally support a small decrease in human colorectal cancer risk with consumption of soy foods (Toyomura & Kono 2002, Spector et al. 2003). We previously reported the decreased incidence of aberrant crypt foci (ACF), pre-neoplastic lesions of the colon, and colon tumors in azoxymethane (AOM)-treated rats fed a soy protein-based diet over their lifetime (Hakkak et al. 2001, Linz et al. 2004). Results from other laboratories demonstrated inhibitory, stimulatory, or no effects of soy protein consumption on colon cancer in rodents (Gee et al. 2000, Toyomura & Kono 2002). Soy isoflavones such as genistein (GEN) and daidzein are known to exhibit anti-tumorigenic actions in vitro at high doses (Yanagihara et al. 1993), although conflicting results in animal models of colon cancer have been reported (Gee et al. 2000, Guo et al. 2004). Biological processes considered to be affected by soy consumption include cell proliferation, apoptosis and cell survival, endocrine signaling, xenobiotic metabolism, and immune system actions (Xiao et al. 2005, Handayani et al. 2006).

Nutritional status affects synthesis and secretion of insulin and insulin-like growth factors (IGFs; Thissen et al. 1994). Elevated levels of insulin and/or IGF-I are associated with increased colorectal cancer risk in humans and rodents (Corpet et al. 1997, Wu et al. 2002, Wei et al. 2005, 2006). Several studies have indicated that soy protein in the diet improves insulin sensitivity and glucose tolerance in rats (Lavigne et al. 2000, Davis et al. 2005). Insulin and IGF-I stimulate glucose uptake via enhanced glucose transporter expression and recruitment in cells (Lane et al. 2002) and glucose influx is associated with enhanced lipogenesis for support of cell proliferation. The ability of Akt to protect such cells from apoptosis depends on the continued availability of glucose (Munoz-Pinedo et al. 2003, Rathmell et al. 2003, Downward 2004), with overexpression of glucose transporters in colon cancer cells linked to the transition to malignancy (Younes et al. 1996, Lamberts et al. 2002). These collective results suggest that changes in circulating insulin and/or IGF-I concentrations and/or enhanced insulin sensitivity of tissues may underlie cancer-related actions of dietary soy protein.
No published study has addressed the effects of dietary soy protein consumed only during pregnancy on development of colorectal tumors in the offspring at the adult stage. Considering the impact of diet and nutritional status during pregnancy on health outcomes of progeny as later adults (Desai & Hales 1997), and the increasing popularity of soy foods, it is important to clarify the consequences, if any, of developmental exposure to soy protein and its components (Badger et al. 2005). In the present work, we have monitored intestinal (small intestine and colon) tumorigenesis in male Sprague–Dawley rats, whose dams consumed soy protein isolate (SPI) or the major soy isoflavone GEN during pregnancy. We report novel associations of dietary SPI during pregnancy with long-lasting (programed) changes in circulating levels of IGF-I and testosterone, expression of colon mRNAs, and tumor multiplicity. Results suggest that the propensity for developing multiple AOM-induced colon tumors may have a fetal component via dietary programing.

Materials and Methods

Diets and animals

Isocaloric, isonitrogenous American Institute of Nutrition (AIN) 93G diets contained casein (CAS) or SPI as sole protein source (200 g/kg diet), or CAS (200 g/kg diet) supplemented with GEN (2.5 g/kg diet, GEN). Casein was from New Zealand Milk Products (North America) Inc. (Santa Rosa, CA, USA). SPI was from The Solae Company (St Louis, MO, USA). GEN was obtained from Sigma–Aldrich. L-Cystine, L-methionine, L-tryptophan, and L-threonine were supplemented to the SPI diet to compensate for differences in levels of these amino acids between CAS and SPI. Vitamins, minerals, corn starch, sucrose, corn oil, and cellulose were added to equivalent levels for all three diets. Diets were formulated by Harlan Teklad (Madison, WI, USA). Pregnant Sprague–Dawley rats from Charles River Laboratories (Wilmington, MA, USA) were received at gestation day 4 and randomly assigned to one of the three dietary groups. Animals were housed in polycarbonate cages containing corncob bedding (1/4" grade; Harlan Teklad) in temperature- and humidity-controlled rooms with a daily photoperiod of 12 h light:12 h darkness. Rats were allowed ad libitum access to food and water and were weighed weekly. Animal use protocols were approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee.

Experimental design

The experimental design is shown in Fig. 1. Immediately after parturition, dams were switched to the CAS diet (represented as CAS, SPI/CAS, and GEN/CAS groups respectively), except for a subgroup of the SPI dams/progeny (chosen at random) in which SPI diet was continued (SPI). At postnatal day (PND) 2, each litter was culled to five males and five females (females were used in an unrelated study). Male offspring were injected subcutaneously with AOM (Midwest Research Institute, Kansas City, MO, USA) in saline, 15 mg/kg body weight (BW) at PND 47 and 55. At this point, 218 male pups entered the experiment (n = 60, 54, 59, and 45 for CAS, SPI, SPI/CAS, and GEN/CAS groups respectively). At 20 weeks after the second AOM administration, animals were euthanized and subjected to tumor evaluation as described previously (Hakkak et al. 2001, Xiao et al. 2006). Serum samples were prepared and stored at −80 °C for later use. Mucosa (no visible tumor tissue included) from the middle third region of colons was harvested by scraping with a glass slide and stored at −80 °C.

Hormone measurements

At gestational day 19, amniotic fluid was collected and pooled for all fetuses of one pregnant rat on each diet. Extraction of isoflavones followed a previously described method (Shelnutt et al. 2002). Total isoflavones were extracted from amniotic fluid that was pre-treated with glucuronidase and sulfatase (Type I, Sigma–Aldrich), while isoflavone aglycones were extracted from untreated amniotic fluid. Quantification of isoflavones was performed on liquid chromatography-mass...
spectrometry (LC–MS) using selected ion monitoring (Shelnutt et al. 2002). Serum insulin and leptin concentrations (at termination of the experiment; Fig. 1) were determined using the rat endocrine LINCoplex kit (Linco Research, St Charles, MO, USA) and Luminex100 system (Luminex, Austin, TX, USA). Assay sensitivities for insulin and leptin were 55.6 and 6.2 pM respectively. The intra-assay variations for insulin and leptin were 3.8 and 10.8% respectively. Serum estradiol was measured using an enzyme immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI, USA). Testosterone was quantified with an EIA kit (Cayman Chemical). The sensitivity of the estradiol assay was 8 pg/ml and intra-assay variation <10-0%. Serum IGF-I was measured using an EIA kit for rat IGF-I (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The assay sensitivity was 30 ng/ml and intra-assay variation <9-1%. Serum IGF-binding protein-1 (IGFBP-1), IGFBP-2, and IGFBP-3 concentrations were measured with ELISA kits (Diagnostic Systems Laboratories). The assay sensitivities for IGFBP-1, IGFBP-2, and IGFBP-3 were 0.25, 0.25, and 0.04 ng/ml respectively. The intra-assay variations were 10.0, 8.7, and 9.7% for IGFBP-1, IGFBP-2, and IGFBP-3 respectively.

Statistical analysis

Statistical computations were performed using SigmaStat for Windows, version 3.11 (SSI, Richmond, CA, USA). The Fisher’s exact test was used to examine diet effects on tumor incidence and tumor pathology. For comparisons of mean values between treatments, t-test or one-way ANOVA with the Holm–Sidak method to adjust for multiple comparisons was used. Statistical significance was set at P≤0.05; a tendency for an effect was indicated for 0.05<P≤0.10.

Results

SIP and colon tumors

The effects of maternally consumed soy protein or GEN during pregnancy on incidence of gastrointestinal cancers in offspring later in life has not previously been reported. To address this question, pregnant Sprague–Dawley rats and their male progeny underwent the experimental protocol depicted in Fig. 1. The GEN/CAS group was included so as to identify effects of SPI that might be due to this isoflavone or alternatively, due to factors in SPI other than this isoflavone. The amount of GEN that was fed resulted in amniotic fluid levels of free and conjugated GEN comparable with those for SPI diet (GEN diet: 15.2 nM free GEN, 49 nM total GEN; SPI diet: 18 nM free GEN, 53 nM total GEN). Additionally,
SPI diet resulted in detectable levels of the soy isoflavone daidzein (conjugated, 60 nM) and its metabolites O-DMA (conjugated, 26 nM) and Equol (free and conjugated, 7 and 169 nM respectively) in rat amniotic fluid at late pregnancy. Diets were isocaloric and isonitrogenous, and similar weights of pups at age PND2 were observed (Fig. 2A). However, early postnatal BW gain of animals in the SPI/CAS group was significantly increased, as BW at PND 11 and PND 18 were greatest for this group of animals (P < 0.05, Fig. 2A). BW of animals lifetime-fed SPI were less than for CAS and SPI/CAS animals, starting from early postnatal life (PND 18, P < 0.05) to termination of the experiment (PND194, P < 0.05, Fig. 2B). Thus, although diets were balanced, differences in type of dietary protein affected postnatal BW accretion.

Male progeny were injected twice with AOM. Tumor status of these animals at 20 weeks post-AOM is summarized in Table 1. Consistent with an earlier report (Hakkak et al. 2001), the SPI group had reduced overall colon tumor incidence, when compared with those lifetime fed CAS (30.2 % vs 56.9%, P < 0.05). The present study localized the protective effect of SPI to the distal one-third region of colon (Table 1). Maternal-only consumption of SPI or GEN had no effect on colon tumor incidence when compared with control CAS diet (Table 1). AOM induces tumors of the small intestine, though at lower frequency than in colon (Xiao et al. 2006). We observed an appreciable number of AOM-induced tumors of the small intestine that almost exclusively resided in the proximal region (duodenum; Table 1). However, there were no effects of diet on the relative incidence of these lesions.

Maternal consumption of SPI or GEN and tumor multiplicity in progeny

To examine effects of diet on colon tumor multiplicity (an index of tumor promotion), the tumor-bearing animals were divided into two categories, single-tumor (one tumor/rat) or multiple-tumor (one tumor/rat) bearing animals. We observed no differences in the relative ratio of single to multiple-tumor-bearing animals between SPI and CAS groups (Fig. 3A). However, maternal consumption of SPI, followed by switch to CAS at delivery, increased the relative percentage of animals with multiple tumors of the colon, when compared with CAS or SPI groups (P < 0.05; Fig. 3A). Interestingly, this effect was not mimicked by the GEN/CAS regimen. Tumor multiplicity for the small intestine was unaffected by diet (Fig. 3B). Diet did not affect average tumor weight in colon or small intestine (data not shown). Tumors were identified as adenomatous polyps (AP) or adenocarcinomas (AC). Hyperplastic lymphoid nodules were also observed by histology but were not classified as tumors. As shown in Fig. 3C, the percentage of colon AC (to total colon AP + AC) was numerically lower in SPI, SPI/CAS, and GEN when compared with CAS; similarly, small intestine tumors of SPI/CAS and GEN/CAS groups had numerically fewer percentage of ACs when compared with CAS (Fig. 3D), although none of these results achieved statistical significance.

Diet effects on circulating hormones and IGF system

At tumor collection, serum insulin concentrations were reduced in the SPI when compared with CAS and GEN/CAS groups (P < 0.05) (Table 2). Maternal-only consumption of SPI or GEN had no effect on serum insulin levels. Serum levels of the adipose tissue-secreted hormone leptin have been suggested as an independent risk factor for human colon cancer (Staton et al. 2003). Interestingly, circulating levels of leptin, like insulin, were decreased in the SPI when compared with other groups (P < 0.05; Table 2). Estrogens and androgens affect the etiology of colon cancer in humans and rodents (Izbicki et al. 1990, Slattery et al. 2005). Serum estrogen levels were low (as expected for male rats) and unaffected by diet regimen. Serum testosterone levels were decreased specifically in the SPI/CAS but not SPI groups (P < 0.05, Table 2). Serum levels of IGF-I and IGF-binding proteins also are potential biomarkers for colon cancer risk (We et al. 2005, 2006). Circulating IGF-I concentrations were increased in the SPI/CAS group when

![Figure 2](https://via.placeholder.com/150)

**Figure 2** Accelerated postnatal growth of SPI/CAS animals. Shown are mean BW for pre-weaning (panel A; PND 2, 11, and 18; n=60, 54, 60, and 45 animals in CAS, SPI, SPI/CAS, and GEN/CAS groups respectively) and at termination of the experiment (panel B; n=58, 53, 56, and 40 animals in CAS, SPI, SPI/CAS, and GEN/CAS groups respectively). aSignificant difference between SPI/CAS and SPI groups (P < 0.05), bSignificant difference between SPI/CAS and CAS groups (P < 0.05), cSignificant difference between CAS and SPI groups (P < 0.05), dSignificantly less BW of SPI than other groups (P < 0.05). Data are means ± S.E.M.
Table 1  Incidence of azoxymethane (AOM)-induced tumors by tissue subsitea

<table>
<thead>
<tr>
<th>Tissue Subsite</th>
<th>CAS</th>
<th>SPI</th>
<th>SPI/CAS</th>
<th>GEN/CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animalsb</td>
<td>58</td>
<td>53</td>
<td>56</td>
<td>41</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>6.9</td>
<td>3.8</td>
<td>0</td>
<td>4.9</td>
</tr>
<tr>
<td>Mid colon</td>
<td>32.8</td>
<td>26.4</td>
<td>41.1</td>
<td>41.5</td>
</tr>
<tr>
<td>Distal colon</td>
<td>37.9</td>
<td>13.2</td>
<td>30.3</td>
<td>36.6†</td>
</tr>
<tr>
<td>Entire colon</td>
<td>56.9†</td>
<td>30.2*</td>
<td>55.4†</td>
<td>63.4†</td>
</tr>
<tr>
<td>Proximal SI</td>
<td>15.5</td>
<td>15.1</td>
<td>14.3</td>
<td>19.5</td>
</tr>
<tr>
<td>Mid SI</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Distal SI</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Entire SI</td>
<td>17.2</td>
<td>15.1</td>
<td>14.3</td>
<td>19.5</td>
</tr>
<tr>
<td>Otherc</td>
<td>1.7</td>
<td>3.8</td>
<td>3.6</td>
<td>4.9</td>
</tr>
</tbody>
</table>

*Difference compared with CAS (P<0.05); †Difference compared with SPI (P<0.05); Fisher's exact test.
^Frequency of tumor-bearing animals (relative to the total number of animals/group); SI, small intestine.
§During the time between AOM treatments and termination of the study, each group lost a small number (1–4) of animals to morbidity or mortality. These animals were omitted from all analyses. The lower number of GEN/CAS animals was the result of fewer numbers of pregnant rat dams placed on this diet; GEN had no effect on pregnancy success.

tumors of the rectum and cecum.

Compared with CAS and SPI groups (P<0.05; Table 2), while the GEN/CAS group had no such increase. Serum IGFBP-1 and IGFBP-3 levels were increased in the SPI, SPI/CAS, and GEN/CAS groups relative to CAS group (P<0.05; Table 2). There were however no diet effects on serum IGFBP-2 concentrations (Table 2). The above results indicate dietary programing of serum IGF-1 and testosterone (CAS versus SPI/CAS) and of serum IGFBP-1 and IGFBP-3 (CAS versus SPI/CAS, GEN/CAS). Interestingly, elevations in IGF-1 and reductions in testosterone were specifically associated with the noted increase in tumor multiplicity for the SPI/CAS rat group.

Colonic gene expression

Enhanced glucose uptake is a hallmark of colon tumors (Haber et al. 1998); the IGF-1 receptor (IGFIR) promotes survival of tumor cells in part, by stimulating glucose uptake and metabolism (Baserga et al. 2003). Interestingly, colonic Igrf1 mRNAs tended to be more abundant in SPI/CAS animals (P=0.064; Fig. 4A). In addition, RNA transcripts encoding GLUT1; SLC2A1, a protein frequently over-expressed in and linked to enhanced proliferation, resistance to therapy, and metastatic propagation of cancer cells (Mayer et al. 2005), exhibited a tendency for an increase in SPI/CAS animals (P=0.076; Fig. 4B).

Focal areas of lymphoid hyperplasia in the colons of SPI-fed rats

The colons of SPI rats had a relatively high frequency of lymphoid nodules (these lesions constituted 25-7% of total presumptive tumors from this group). Incidence of this pathology was 10-9, 2-7, and 1-8% in GEN/CAS, SPI/CAS, and CAS groups respectively. These focal areas of hyperplasia displayed an infiltrate of lymphocytes in the submucosa and lamina propria, without invasion into the muscular. B cell-rich follicular centers (CD79a positive) surrounded by expanded parafollicular T cell-rich regions (CD3 positive) were observed to comprise these nodules associated with lifetime SPI consumption in this model.

Discussion

The ‘fetal origins of adult disease’ hypothesis refers to conditions in which metabolic, nutritional, or hormonal perturbations during critical periods of fetal or postnatal development elicit persistent effects on health during later adulthood (reviewed in Desai & Hales 1997). This hypothesis stimulated our efforts to examine whether physiological effects of SPI could be conveyed to offspring via maternal dietary exposure during gestation. Our results show that maternal-only SPI exposure is quite distinct from lifelong SPI diet in the effects elicited. While we observed no differences in tumor incidence for CAS, SPI/CAS, and GEN/CAS groups, tumor multiplicity was increased in the SPI/CAS group. This latter result likely reflects activation of signaling and/or metabolic pathway(s) that favor promotion of initiated tumor cells, with the nutritional shift from SPI to CAS at delivery as a primary stimulus. The lack of a similar response for GEN suggests that other component(s) of SPI may be responsible for this effect.

The peripubertal increase in circulating IGF-1 in rats is known to be affected by developmental or nutritional programing occurring in prenatal or very early postnatal periods (Handelsman et al. 1987). In agreement with this, SPI/CAS animals had heavier BW during postnatal growth and increased serum IGF-1 at termination of the experiment. IGF-I circulates in complex with IGFBPs that sequester IGFs and also serve as tissue/cell targeting proteins for these ligands. CAS sera were notable in that they had reduced levels of IGFBP-1 and IGFBP-3, two of the major serum carriers when compared with all other diet groups. Moreover, levels of IGFBP-3, a protein that is inversely associated with human colon cancer risk (Renehan et al. 2001), were lower in SPI/CAS than SPI groups. These data point to the likelihood of increased tumor incidence and multiplicity respectively of these animals. Another hormone, whose circulating levels exhibited evidence of programing by maternal SPI, but not GEN, was testosterone. Unlike IGF-I, this hormone was decreased in the circulation, which also may be of physiological significance, as testosterone is tumor suppressive in AOM-treated rats (Izbicki et al. 1990). Dietary GEN during pregnancy elicited long-lasting (i.e., programed) increases in circulating IGFBP-1 and IGFBP-3; with the exception of IGFBP-3, these effects followed that for the SPI and SPI/CAS groups. Therefore, with respect to these specific indices, dietary GEN during pregnancy recapitulated the effects of SPI during pregnancy and of the lifetime SPI diet.
The present study confirmed that lifelong consumption of SPI reduces AOM-induced colon tumor incidence in male Sprague–Dawley rats (Hakkak et al. 2001, Toyomura & Kono 2002, Linz et al. 2004). This animal model may mimic the traditional Asian population who consume soy foods throughout life and have reduced incidence of colon cancers when compared with those consuming a ‘Western’ diet (Lee et al. 1989, Badger et al. 2005). The diminutions in serum insulin and leptin concentrations are of particular interest in this regard. Soy protein in the diet enhances insulin sensitivity in rats resulting in

Table 2 Effects of diet on circulating hormone and insulin-like growth factor-binding protein (IGFBP) levels (mean ± S.E.M.)*

<table>
<thead>
<tr>
<th></th>
<th>CAS</th>
<th>SPI</th>
<th>SPI/CAS</th>
<th>GEN/CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pM)</td>
<td>313.8 ± 23.3</td>
<td>225.4 ± 22.1</td>
<td>272.9 ± 26.7</td>
<td>343.2 ± 35.5</td>
</tr>
<tr>
<td>Leptin (pM)</td>
<td>393.5 ± 20.3</td>
<td>285.6 ± 24.8</td>
<td>382.3 ± 23.2</td>
<td>371.4 ± 27.6</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>2125.5 ± 107.8</td>
<td>2067.9 ± 100.3</td>
<td>2499.6 ± 125.3</td>
<td>2222.9 ± 175.2</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>0.09 ± 0.047</td>
<td>0.04 ± 0.047</td>
<td>0.38 ± 0.059</td>
<td>0.32 ± 0.052</td>
</tr>
<tr>
<td>IGFBP-2 (ng/ml)</td>
<td>37.1 ± 4.4</td>
<td>39.4 ± 3.1</td>
<td>43.5 ± 3.3</td>
<td>42.0 ± 1.6</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>64.6 ± 5.4</td>
<td>179.8 ± 13.2</td>
<td>127.5 ± 9.5</td>
<td>222.4 ± 12.2</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>14.7 ± 2.9</td>
<td>14.8 ± 2.6</td>
<td>10.4 ± 1.7</td>
<td>12.9 ± 2.7</td>
</tr>
<tr>
<td>Testosterone (pg/ml)</td>
<td>495.7 ± 74.8</td>
<td>448.0 ± 94.8</td>
<td>234.9 ± 40.4</td>
<td>380.2 ± 112.2</td>
</tr>
</tbody>
</table>

*Data for tumor-bearing and tumor-free animals, at 20 week post-AOM, were combined since two-way ANOVA indicated no effect of tumor status on circulating levels of each of the above hormones (P>0.05); n=10 animals/group for insulin, leptin, and IGF-I; n=9 animals/group for IGFBP-1, IGFBP-2, IGFBP-3, estradiol, and testosterone assays. †Difference with CAS (P<0.05), ‡Difference with SPI (P<0.05), §Difference with SPI/CAS (P<0.05); t-test.
GLUT1 is typical for human colon tumors (Haber et al. 2000, Linz et al. 2004, Davis et al. 2005). The lower leptin levels for our SPI rats likely reflects this decrease in adiposity, since body composition data from our laboratory demonstrated lower total body fat content of SPI-fed, AOM-treated male rats (Linz et al. 2004). Others have reported that hyperinsulinemia is a major risk factor for human colon cancer and that exogenous insulin or a state of insulin resistance can promote colon tumorigenesis in the AOM-rat model (Corpet et al. 1997, Tran et al. 2003, Ma et al. 2004). Leptin is a mitogen for human HT29 colon cancer cells (Liu et al. 2001) and serum levels of this protein are a risk factor for colorectal cancer in men (Stattin et al. 2003). Thus, we suggest that the lifetime SPI diet is protective against cancer initiation in the distal regions of the colon by virtue of its hypo-insulinemic, insulin-sensitizing, and anti-adipogenic actions. Direct effects of SPI isoflavones to inhibit proliferation of initiated tumor cells (Yanagihara et al. 1993, Booth et al. 1999) also may contribute to the overall protective effects of the lifetime SPI diet, which would not have been observed in the SPI/CAS or GEN/CAS diet treatment paradigms used here. A molecular explanation for why SPI inhibited distal colon but not mid- or proximal colon or small intestine tumor incidences remains to be addressed.

We observed no changes in the circulating levels of insulin in SPI/CAS and GEN/CAS, relative to CAS animals, suggesting that these dietary paradigms did not program insulin resistance and hyperinsulinemia during AOM-induced carcinogenesis. Instead, colon Igf1r and Slc2a1 (Glut1) mRNAs tended to be up-regulated by maternal-only SPI. Up-regulated expression of Glut1 is typical for human colon tumors (Haber et al. 1998) to support their growth via enhanced utilization of glucose in glycolysis (Munoz-Pinedo et al. 2003). The tendency for increased Glut1 expression in colon tissue of the SPI/CAS animals suggests positive relationships with elevated serum IGF-I, tumor multiplicity, and mucosal ACFs (the latter lesions were not measured here but undoubtedly were present in the non-tumor colon mucosal tissue). The observation that colon tumor incidence and tumor multiplicity in the GEN/CAS and CAS groups did not differ, whereas the two diet groups had markedly different levels of circulating IGFBP-1 and IGFBP-3 leaves open the putative roles of these proteins and their downstream pathways in colon tumorigenesis.

The occurrence of hyperplastic lymphoid nodules in colons of lifetime SPI-fed animals is interesting. These lymphoid hyperplasias in colonic submucosa and lamina propria were florid in nature. Although the infiltrate appeared monomorphic in hematoxylin-stained sections, immunohistochemistry for CD3 and CD79a revealed B cell-rich follicular areas surrounded by expanded parafollicular T cell-rich regions. Soy protein and GEN diets differentially affect gastrointestinal and systemic immune functions (Yellayi et al. 2002, Xiao et al. 2005, Cooke et al. 2006). A possible link between Type 1 diabetes, mild or atypical celiac disease, and gastrointestinal lymphoid pathology has been described (O’Connor et al. 1999). Thus, future studies will evaluate whether lifelong suppression of circulating insulin may have predisposed SPI-fed rats to attendant increased occurrence of colon lymphoid nodules.

In summary, dietary exposure to a soy protein–based diet during pregnancy followed by the switch to CAS at delivery increased colon tumor multiplicity (a measure of tumor promotion) in the male progeny as later adults, as well as permanently altered several endocrine parameters previously linked to colon carcinogenesis. The present results raise the possibility that colon cancer, which conventionally is considered to be a cancer of the elderly, may be influenced by dietary/metabolic perturbations or programing occurring during development. Epidemiological studies that address maternal nutritional status and diet during pregnancy, along with that for progeny, are required to examine this potentially important question in the human population. Lastly, colon tissue glucose uptake may be subject to dietary programing during development and this potentially may underlie in part, colon cancer-stimulatory or –inhibitory signals of dietary proteins.

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