JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Combined Consumption of Soy Germ and Tomato Powders Results in Altered Isoflavone and Carotenoid Bioavailability in Rats

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ABSTRACT: The efficacy of combinations of food for enhanced anticancer activity is of clinical interest, but there is limited information on the effect of combined consumption on bioactive bioavailability. Male Copenhagen rats consumed diets containing 10% tomato powder (TP), 2% soy germ (SG), neither, or a combination (TP+SG) for 25 weeks (n = 63) or 7 days (n = 24). After 7 days, serum carotenoids were significantly lower after TP+SG feeding compared to TP alone. After 25 weeks, the TP+SG group had significantly lower lycopene and β-carotene concentration in the testes, seminal vesicles, and ventral prostate compared to the TP group and significantly higher urinary isoflavone excretion compared to the SG group. These differences were not explained by mRNA expression of scavenger receptor class B type I, carotene 15,15′-monooxygenase I, carotene 9′,10′-monooxygenase II, or activity of hepatic detoxification enzymes. The results suggest interactions between soy germ and tomato powder that enhance isoflavone absorption but reduce carotenoid bioavailability.

KEYWORDS: tomato, soy, isoflavones, carotenoids, prostate cancer, bioavailability

INTRODUCTION

A diet containing a variety of fruits and vegetables is recommended to reduce the risk of chronic disease. Numerous foods and bioactive compounds have shown chemopreventive potential against prostate cancer (PCa),[1] the most diagnosed cancer in U.S. men.[2] A growing body of evidence suggests that consumption of soy or tomato products is associated with a reduced risk of PCa.[3,4] Soy is a rich source of bioactives including isoflavones, saponins, and lignans. Research suggests soy isoflavones may inhibit proliferation, increase apoptosis, inhibit tumor growth and angiogenesis, be anti-inflammatory, and modulate steroid hormone levels and receptors in the prostate.[5] Tomatoes are also of interest for the prevention of PCa due to the anticarcinogenic properties of their nutrients and phytochemicals, primarily polyphenols and carotenoids such as lycopene, phytoene, phytofluene, and β-carotene.[6] Tomato phytochemicals have been suggested to induce cell-cycle arrest, induce phase II enzymes, function as antioxidants, modulate growth factor signaling, and increase cell−cell communication via gap junctions.[6,7] Typically, tomato and soy products are not consumed together; however, the use of combinations of these food and their bioactives has gained interest for the prevention of PCa.[8,9] Whole foods and combinations of foods and bioactives may be more protective due to the presence of multiple phytochemicals that may have additive or synergistic bioactivity;[10−12] however, not all combinations of foods or bioactives will be beneficial. PCa patients with rising prostate-specific antigen (PSA) were randomized to receive supplements containing 15 mg of lycopene or 15 mg of lycopene plus 40 mg of soy isoflavones twice daily for 6 months. Lycopene supplementation was more effective in reducing serum PSA progression than the combined supplementation, suggesting a negative interaction between lycopene and soy isoflavones.[9]

The efficacy of combinations of foods and bioactives for enhanced health benefits is of clinical interest, but there is limited information on the interactions between foods that affect bioavailability or bioactivity of phytochemicals. The purpose of this study was to measure the bioavailability of soy germ isoflavones and tomato carotenoids in male Copenhagen rats after individual and combined consumption of soy germ and tomato powder. Exposure to bioactives was estimated by carotenoid bioaccumulation and urinary isoflavones. The effects of diet on the expression of scavenger receptor class B type I (SR-BI) (a protein involved in carotenoid absorption[13]) and the carotenoid metabolizing enzymes carotenoid 15,15′-monooxygenase (CMOI) and carotenoid 9′,10′-monooxygenase II (CMOII) were measured in prostate, liver, and duodenal mucosa.

MATERIALS AND METHODS

Animals and Experimental Design. The University of Illinois Laboratory Animal Care Advisory Committee approved all animal procedures.

Short-Term Feeding. Twenty-four male Copenhagen rats (Cop 2331; Harlan, Indianapolis, IN) were acquired at ∼34 weeks of age, housed individually in wire-bottom cages, and acclimated to powdered AIN-93G diet for 1 week. Animals were then randomized (n = 6/group) to consume AIN-93G-based experimental diets for 7 days. Experimental diets were AIN-93G control, 10% whole tomato powder (FutureCeuticals, Momence, IL), 2% soy germ powder (Frutarom, North Bergen, NJ), and 10% tomato powder with 2% soy germ powder. Soy germ, the hypocotelydon of soy, is separated during milling of whole soybeans and is a concentrated source of soy bioactives including isoflavones, saponins, and phytosterols. Soy germ has a unique isoflavone profile with about 59% daidzein, 34% glycitein, and 7% genistein. In contrast, the typical isoflavone profile of soy products that utilize the whole soybean is approximately 40% daidzein, 10% glycitein, and 50%

Received: January 28, 2011
Revised: March 18, 2011
Accepted: March 30, 2011
Published: March 30, 2011

Table 1. Composition of Experimental Diets

	g/100 g total diet			
	control	10% tomato powder[a]	2% soy germ[b]	10% tomato powder + 2% soy germ[a,b]
cornstarch	39.7	35.2	39.7	35.2
casein	20.0	18.5	19.1	17.6
maltodextrin	13.2	11.8	13.2	11.8
sucrose	10.0	10.0	10.0	10.0
fiber[c]	5.0	3.3	4.4	2.7
mineral mix[d]	3.5	3.5	3.5	3.5
vitamin mix[e]	1.0	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3	0.3
choline bitrate	0.3	0.3	0.3	0.3
cottonseed oil	7.0	6.5	6.6	6.2
tomato powder[f]		10.0		10.0
soy germ[g]			2.0	2.0

[a] Short-term study diet contains 41 nmol of lycopene, 0.9 nmol of β-carotene, 11.9 nmol of phytoene, and 3.7 nmol of phytofluene per gram of diet. Long-term study diet contains 149 nmol of lycopene, 1.5 nmol of β-carotene, 6.4 nmol of phytoene, and 1.8 nmol of phytofluene per gram of diet. [b] Contains 670 nmol of total daidzein, 387 nmol of total glycitein, and 74 nmol of total genistein per gram of diet. [c] Non-nutritive cellulose. [d] AIN93-MX formulation. [e] AIN93-VX formulation. [f] FutureCeuticals "HiActives" Tomato Powder 2000 N8. [g] Frutarom SoyLife Complex Micro.

genistein. Diets were balanced for protein, fat, energy, and fiber, and stored at 4 °C in the dark (Table 1). Diets were made with cottonseed oil rather than soybean oil to minimize exogenous isoflavones. Rats were weighed weekly, and individual feed intake was measured every 48 h when fresh diet was provided.

Long-Term Feeding. Sixty-three male Copenhagen rats (COP/NCrl; Charles River, Wilmington, MA) were obtained at ∼6 weeks of age and individually housed in wire-bottom cages. After 1 week of adaptation to powdered AIN-93G diet, animals were randomly assigned to consume AIN-93G control (n = 17), 10% whole tomato powder (n =17), 2% soy germ powder (n = 16), or 10% tomato powder with 2% soy germ powder (n = 13) for 25 weeks. Due to seasonal and batch variations, the carotenoid content of the tomato powders differed between the long-term and short-term studies (Table 1).

Carotenoid Extraction and Quantification. Carotenoid extraction was performed using previously published techniques described by our laboratory.[14,15] General precautions for work with carotenoids were taken throughout the extraction procedure. Briefly, 5 mL of ethanol solution containing 0.01% BHT was added to tissue or 25 mg of diet. Adrenals, ventral prostate, seminal vesicles, and testes were pooled within groups to facilitate HPLC detection of carotenoids, and liver and serum were extracted in duplicate. Tissues were minced thoroughly, and diet was homogenized for 75 s. Samples were saponified with 1 mL of saturated KOH for 30 min at 60 °C. Diet and tissue samples were extracted three times with 6 mL of hexane. Three hundred microliters of an ethanol/BHT solution was added to 300 μL of serum and extracted three times with 1.5 mL of hexane. Extracts were dried in a Speedvac (model AS160; Savant, Farmingdale, NY) evaporator, flushed with argon, and stored at −20 °C for ≤48 h before reverse-phase HPLC-PDA analysis as previously described.[15] Analytical standards of lycopene, phytoene, phytofluene, and β-carotene were used for quantification. An analytical standard for lycopene was extracted from Redvivo 10% lycopene beadlets (gift from DSM Nutritional Products, Kaiseraugst, Switzerland), and phytoene, phytofluene, and β-carotene analytical standards were gifts from Hansgeorg Ernst from BASF (Ludwigshafen, Germany). Our laboratory participates in the National Institute of Standards and Technology Fat Soluble Vitamin Round Robin Program, and our lycopene analysis is consistently within one standard deviation of the Round Robin median/expected value.

Urinary Isoflavone Quantification. In the short -term feeding, 24−48 h before study termination, rats were randomized to metabolic cages for a 24 h urine collection. Urine was hydrolyzed with β-glucuronidase (from *Escherichia coli* K12, 200 U/mL at 25 °C; Roche Diagnostics, Indianapolis, IN) and arylsulfatase (from *Helix pomatia* 24190 U/g; 2 mg/mL in 1 M (pH 6.0) acetate buffer; type H-1, Sigma, St. Louis, MO) for 1 h at 37 °C. Incubates were extracted with 1.5 mL of methyl *tert*-butyl ether and evaporated to dryness. During the final week of feeding in the long-term study, rats were randomized to metabolic cages for 24-h urine collection. Urine was hydrolyzed with β-glucuronidase and sulfatase (from *H. pomatia*; 500 U/mL in 1 M (pH 5.0) acetate buffer; type H-1, Sigma) for 16 h at 37 °C. Incubates were extracted three times with 2 mL of diethyl ether and evaporated to dryness. In both studies, $^{13}C_3$-daidzein, equol, *O*-desmethylangolensin (DMA), and $^{2}H_4$-genistein (provided by Cancer Research Center of Hawaii) were added as internal standards, and extracted samples were resuspended in 1:1 methanol/0.1% aqueous formic acid and analyzed for genistein, daidzein, glycitein, and equol by LC-MS as previously described[16] at the Cancer Research Center of Hawaii analytical laboratory.

Isolation of Hepatic Microsomal and Cytosolic Fractions. A 300-mg sample of liver was homogenized in 1 mL of 0.05 M Tris-HCl buffer (pH 7.4) and centrifuged at 10000*g* for 20 min at 4 °C. The microsomal and cytosolic fractions were separated by centrifugation of the supernatant at 100000*g* for 60 min at 4 °C. The microsomal fraction was resuspended in 1 mL of 0.05 M Tris-HCl buffer (pH 7.4) with 0.25 M sucrose. Both fractions were snap frozen in liquid nitrogen and stored at −80 °C until further use.

Enzyme Activity. Cytochrome P450 1A (CYP1A) activity in the microsomal fraction and NQO1 activity in the cytosolic fraction were measured as previously described.[14] Briefly, the formation of resorufin from ethoxyresorufin by ethoxyresorufin *O*-deethylase (EROD) was measured in an FLx800 plate reader (BioTek Instruments, Winooski, VT) over 3 min and calculated using a resorufin standard curve. CYP1A activity is correlated with EROD activity; therefore, the specific activity of CYP1A was calculated as picomoles of resorufin per minute per milligram of protein. NAD(P)H-quionone oxidoreductase 1 (NQO1) in the cytosolic fraction was measured as nanomoles of MTT reduced per minute per milligram of protein. In the reaction mixture, NQO1 reduces menadione to menadiol, and the amount of MTT reduced by menadiol (colorimetric assay) was quantified in a microplate absorbance reader.

Real Time Quantitative PCR. At time of tissue harvest, duodenal mucosa, dorsolateral prostate, and a section of liver was placed in RNA*later* solution (Ambion, Austin, TX) and stored at 4 °C for 24 h. Tissues were then blotted dry and transferred to a fresh tube for storage at −80 °C until analysis. RNA was extracted from tissues with Trizol (Invitrogen, Carlsbad, CA), DNase treated (New England Biolabs, Ipswich, MA), and synthesized into cDNA by a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). mRNA expression of carotenoid 15,15′-monooxygenase I (NM_053648), forward 5′-CCTAGAGCTCCCTCGGATAAATTA and reverse 5′-GTTGGGACTGGACTCCATTGTACT-3′; carotenoid 9′, 10′-monooxygenase II (DQ083174), forward 5′-ACCATCTCCCAGTTTTGAAGAAC-3′ and reverse 5′-GCAATGCACGGCAGACTCT-3′; SR-BI (NM_01541.1), forward 5′-CTGGTGCCCATCATTTACCAA-3′ and reverse 5′-AGCCCTTTTTACTACCACTCCAAA-3′; and ribosomal protein L7a (RPL7a, NM_001114391) forward 5′-GAGGCCAAAAAGGTGGTCAATCC-3′ and reverse

Table 2. Body Weight and Feed Intake in 7-Day and 25-Week Feeding Studies[a]

diet	AIN-93G	10% tomato powder	2% soy germ	10% tomato powder + 2% soy germ
		7-Day Feeding Study		
starting weight (g)	366 ± 12	366 ± 10	367 ± 10	360 ± 11
ending weight (g)	383 ± 11	381 ± 12	382 ± 10	378 ± 8
total feed intake (g)	152 ± 4	140 ± 7	138 ± 3	150 ± 9
gain/feed (g/g)	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.02
		25-Week Feeding Study		
starting weight (g)	159 ± 2	160 ± 2	161 ± 2	159 ± 3
ending weight (g)	403 ± 6 ab	420 ± 6 b	399 ± 6 a	388 ± 6 a
total feed intake (g)	2740 ± 30	2740 ± 40	2790 ± 50	2710 ± 40
gain/feed ratio (g/g)	0.089 ± 0.002 ab	0.095 ± 0.002 b	0.085 ± 0.002 a	0.084 ± 0.001 a

[a] Values are the mean ± SEM. Different letters within a row indicate significant differences between treatments ($p < 0.05$).

5′-CCTGCCCAATGCCGAAGTTCT-3′ was measured by real-time PCR using SYBR Green Master Mix (Applied Biosystems, Foster City, CA) and monitored by an ABI Prism 7900HT (Applied Biosystems). RPL7a was used as a housekeeping gene, and relative mRNA abundance was determined using the comparative critical threshold method.

Hepatic Lipid Content and Serum Cholesterol. Serum total cholesterol was measured in duplicate with a diagnostic Cholesterol E Kit (Wako Chemicals, Richmond, VA). Liver lipids were extracted in duplicate using a modified Folch method as previously described.[17]

Statistical Analysis. Data are shown as the mean ± SEM. Differences in carotenoid concentration and urinary isoflavones were analyzed by a Student *t* test and considered to be significant at $p < 0.05$. Differences among treatment groups for weight, feed intake, and gain/feed ratio were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey−Kramers studentized range test with $\alpha < 0.05$ when the assumptions of ANOVA were met; otherwise, the Wilcoxon and Kruskal−Wallis nonparametric tests were used. Differences among treatment groups for mRNA expression, hepatic lipids, serum cholesterol, and enzyme activity were analyzed by one-way ANOVA followed by orthogonal contrast tests for a priori hypotheses. All statistical analyses were conducted with SAS (version 9.2; SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Weight Gain and Feed Intake. All diets were readily consumed, and there were no differences in feed intake between diet groups in either the long-term or short-term studies (Table 2). However, in the long-term study, the TP+SG group gained significantly less weight than the TP group, and the gain/feed ratios of the SG and TP+SG groups were significantly lower than that of the TP group. All diets were isocaloric and matched in macronutrients; therefore, the differences in weight gain were unexpected. Previous rodent studies have shown significant effects on weight gain and fat mass by dietary supplementation with soy or its phytoestrogens, suggesting that soy or its bioactives may alter metabolism by increasing energy expenditure and activating fatty acid oxidation.[18] The metabolic effects of dietary soy germ in rats have never been investigated, and the reduced weight gain by long-term feeding of soy germ suggests that the experimental diets contained enough soy isoflavones to modulate energy utilization compared to the tomato powder group.

Serum and Tissue Carotenoids. In the short-term study, the TP+SG group had significantly lower serum levels of phytoene

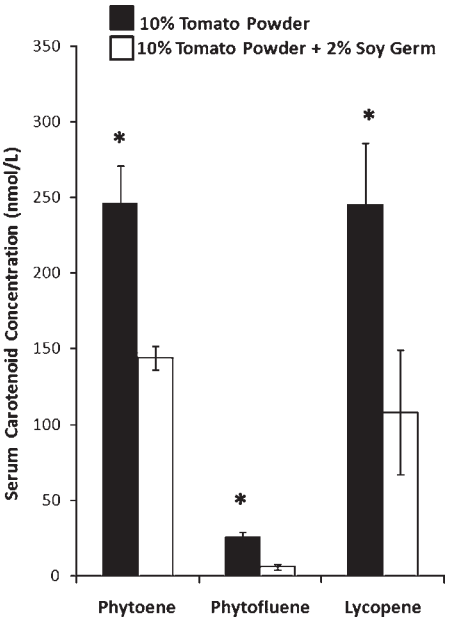

Figure 1. Serum carotenoids in 7-day feeding study. Values are the mean ± SEM, $n = 6$. ∗ indicates significant difference between treatments ($p < 0.05$). β-Carotene was below detection limits.

(−42%, $p = 0.01$), phytofluene (−77%, $p < 0.001$), and lycopene (−56%, $p = 0.02$) than the TP group (Figure 1), which suggests that soy germ may alter the initial absorption and/or metabolism of carotenoids. Long-term consumption of TP+SG did not result in a significant reduction in serum carotenoids compared to the TP group (Table 3); however, there was a general reduction in carotenoid bioaccumulation in rats fed TP + SG compared to TP alone. Lycopene and β-carotene concentrations in the testes ($p = 0.001$, $p = 0.003$), seminal vesicles ($p = 0.04$, $p = 0.003$), and ventral prostate ($p = 0.002$, $p = 0.001$) were significantly lower in the TP+SG group than in the TP group. Carotenoid saturation of lipoproteins in the 25-week feeding, but not in the 7-day study, may explain why there were no significant differences in serum carotenoids between the TP and TP+SG groups in the 25-week study. Carotenoids are transported in the blood by lipoproteins, and studies suggest that serum carotenoid concentrations eventually plateau. A previous rat study in our laboratory showed that serum lycopene levels were not

Table 3. Serum and Tissue Carotenoid Concentration in 25-Week Feeding[a]

carotenoid	10% tomato powder	10% tomato powder + 2% soy germ
Serum (nmol/L)		
phytoene	262 ± 18	193 ± 29
phytofluene	113 ± 20	92 ± 17
lycopene	512 ± 96	479 ± 120
β-carotene	ND[b]	ND
Liver (nmol/g)		
phytoene	29.2 ± 1.8 a	19.5 ± 0.9 b
phytofluene	55.3 ± 6.4 a	36.1 ± 2.9 b
lycopene	141 ± 19	103 ± 7
β-carotene	ND	ND
Adrenals (nmol/g)		
phytoene	24.9 ± 3.2	18.4 ± 1.2
phytofluene	28.5 ± 4.0	19.6 ± 1.4
lycopene	19.8 ± 2.6	13.7 ± 0.8
β-carotene	0.30 ± 0.03 a	0.19 ± 0.01 b
Testes (nmol/g)		
phytoene	0.06 ± 0.01	0.05 ± 0.01
phytofluene	0.19 ± 0.03	0.16 ± 0.02
lycopene	1.04 ± 0.07 a	0.59 ± 0.08 b
β-carotene	0.010 ± 0.001 a	0.006 ± 0.001 b
Seminal Vesicles (nmol/g)		
phytoene	ND	ND
phytofluene	0.026 ± 0.001 a	0.018 ± 0.001 b
lycopene	0.22 ± 0.01 a	0.19 ± 0.01 b
β-carotene	0.0038 ± 0.0001 a	0.0030 ± 0.0001 b
Ventral Prostate (nmol/g)		
phytoene	ND	ND
phytofluene	0.058 ± 0.003 a	0.043 ± 0.002 b
lycopene	0.53 ± 0.02 a	0.38 ± 0.01 b
β-carotene	0.0095 ± 0.0003 a	0.0065 ± 0.0003 b

[a] Values are the mean ± SEM. Liver, serum, and testes, $n = 7$; adrenals $n = 3$; seminal vesicles and ventral prostate $n = 3-4$. Different letters within a row indicate significant differences between treatments ($p < 0.05$). [b] ND, carotenoid levels below detection limit.

significantly different between animals receiving dietary lycopene supplementation of 0.05 or 0.50 g/kg diet after 8 weeks,[19] suggesting a saturation point in the blood. In men with prostate cancer, lycopene supplementation resulted in increased plasma lycopene levels that reached a plateau at 3 months and remained stable for the duration of the 12-month intervention.[20] Differences in tissue carotenoids but not serum carotenoids in the long-term study suggest that in long-term consumption, serum carotenoids may reach a plateau and may not be an accurate indicator of tissue carotenoid bioavailability.

CMOI, CMOII, and SR-BI mRNA Expression. The differences in tissue carotenoid bioaccumulation in the long-term feeding suggest that soy germ may alter carotenoid uptake and/or metabolism. Carotenoid 15,15′-monooxygenase (CMOI) centrally cleaves provitamin A carotenoids to retinal. Carotenoid 9′,10′ monooxygenase II (CMOII) can eccentrically cleave β-carotene to β-apocarotenals,[21] and in vivo and in vitro evidence suggests that it may cleave lycopene.[22,23] Compared to the control group, hepatic CMOI expression was significantly higher in rats consuming 10% tomato powder for 25 weeks, but there was no up-regulation in the TP+SG group (Table 4), suggesting potential differences in bioactivity of these dietary interventions. There were no other alterations by diet in CMOI or CMOII expression in the liver, duodenal mucosa, or dorsolateral prostate. In vitro studies suggest that dietary flavonoids, such as the soy isoflavone genistein, may alter CMOI activity;[24] therefore, potential changes in enzyme activities by soy germ are of further interest.

Carotenoids must be incorporated into mixed micelles composed of bile salts and dietary lipids before absorption into the enterocyte and subsequent secretion into the lymph via chylomicrons.[13] Absorption into the enterocyte may be through simple diffusion or receptor-mediated transport such as through scavenger receptor, class B, type I (SR-BI).[13] The expression of SR-BI was measured but was not significantly altered by diet in duodenal mucosa, liver, or dorsolateral prostate, suggesting that differences in carotenoid bioaccumulation were not through differences in carotenoid transport. However, carotenoids may also be absorbed by passive diffusion or other transporters,[25] which were not measured.

Hepatic Lipids and Serum Cholesterol. Due to the lipophilic nature of carotenoids, the absorption and subsequent bioavailability are dependent on factors that may affect micellerization of carotenoids such as the amount of dietary fat in the meal or the presence of sterols, stanols, or fiber.[13] Soy germ diets contained 1 mg/kg sterols and stanols (analysis provided by supplier), and reduced carotenoid absorption by sterols and stanols has also been correlated with reduced LDL cholesterol levels.[13] There were no significant differences in total hepatic lipid accumulation (36.2 ± 5.2) or total serum cholesterol (160.0 ± 22.2) between any of the groups, suggesting that differences in carotenoid bioaccumulation by dietary soy germ were not likely due to effects on intestinal micellerization or lipid absorption. A limitation of the study is that only total serum cholesterol was measured; therefore, dietary influences on individual lipoproteins were not able to be identified.

Urinary Soy Isoflavones. Isoflavones in foods exist primarily as glycosides and must be hydrolyzed by β-glucosidases on the brush border membrane or by bacterial β-glucosidases in the intestine before absorption. Aglycones can then enter the enterocyte via passive diffusion to be glycosylated, sulfated, or glucuronidated.[26] In the liver, conjugated isoflavones go through first-pass metabolism and can be excreted back into the intestine via the bile. In the intestine, isoflavones undergo deconjugation for reabsorption or undergo further metabolism by the intestinal microflora to form metabolites such as *O*-desmethylangolensin and equol.[26] Isoflavone absorption and metabolism are highly dependent on intestinal microflora, but other factors such as dose, dietary habits, and the food matrix may affect bioavailability.[27] In the short-term study, urinary excretion of glycitein was significantly higher in the SG group than in the TP+SG group, but there were no other significant differences between groups (Table 5). In the long-term study, urinary total isoflavones, daidzein and equol, were significantly higher in the TP+SG group than in the SG group. Feed intakes between diet groups were not significantly different; therefore, the data suggest that long-term consumption of tomato powder may enhance isoflavone absorption and/or equol production.

Hepatic Detoxification Enzyme Activity. The isoflavones daidzein, genistein, and glycitein are known substrates of CYP450

Table 4. Hepatic, Intestinal, and Prostatic mRNA Expression of CMOI, CMOII, and SR-BI in 25-Week Feeding Study [a]

	fold change			
tissue	AIN-93G	10% tomato powder	2% soy germ	10% tomato powder + 2% soy germ
		CMOI		
liver	1.0 ± 0.1 a	1.9 ± 0.4 b	1.4 ± 0.1 ab	1.4 ± 0.1 ab
duodenal mucosa	1.0 ± 0.3	1.1 ± 0.2	0.8 ± 0.2	0.7 ± 0.1
dorsolateral prostate	ND[b]	ND	ND	ND
		CMOII		
liver	1.0 ± 0.1	1.3 ± 0.3	1.1 ± 0.2	0.9 ± 0.1
duodenal mucosa	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1
dorsolateral prostate	1.0 ± 0.2	2.0 ± 0.9	0.9 ± 0.2	1.9 ± 0.9
		SR-BI		
liver	1.0 ± 0.2	1.5 ± 0.4	2.6 ± 0.9	2.1 ± 0.6
duodenal mucosa	1.0 ± 0.2	1.3 ± 0.3	1.0 ± 0.2	0.8 ± 0.1
dorsolateral prostate	1.0 ± 0.3	1.0 ± 0.3	0.8 ± 0.2	0.7 ± 0.1

[a] Values reported as relative to expression in control (AIN-93G), which was set to 1. Results shown are the mean ± SEM, $n = 7-11$. Different letters within rows indicate significant differences between treatments ($p < 0.05$). [b] ND, expression was not detectable.

Table 5. 24-Hour Urinary Isoflavone Excretion in 7-Day and 25-Week Feedings [a]

	μmol of isoflavone/L of urine	
	2% soy germ	10% tomato powder + 2% soy germ
	7-Day Feeding	
daidzein	184 ± 23	131 ± 11
genistein	14 ± 2	12 ± 1
glycitein	34 ± 6 a	16 ± 1 b
equol	267 ± 15	259 ± 29
total isoflavones	499 ± 43	418 ± 37
	25-Week Feeding	
daidzein	157 ± 26 a	272 ± 34 b
genistein	19 ± 2	23 ± 2
glycitein	67 ± 17	116 ± 25
equol	413 ± 51 a	615 ± 54 b
total isoflavones	656 ± 88 a	1025 ± 107 b

[a] Values are the mean ± SEM $n = 5-6$. Different letters within rows indicate significant differences between treatments ($p < 0.05$). Total isoflavones = daidzein + glycitein + genistein + equol. Isoflavones were not detectable in urine from rats that consumed AIN-93G or 10% tomato powder.

enzymes;[28,29] therefore, the different urinary isoflavone profiles between the SG and TP+SG groups may be explained by differences in hepatic metabolism by phase I and II enzymes. However, diet had no effect on hepatic NQO1 enzyme activity in the long-term and short-term studies, and hepatic EROD activity was not altered by diet in the long-term study. In the short-term study, hepatic EROD activity was 1.6 times higher in rats that consumed TP+SG compared to SG alone ($p < 0.01$). Higher hepatic EROD activity in the TP+SG group may indicate increased isoflavone metabolism, partially explaining the general trend of lower urinary isoflavone excretion compared to the SG group in the short-term feeding.

A possible explanation for the increase in urinary isoflavones after long-term consumption of TP+SG may be increased isoflavone absorption in the gut facilitated by gut microflora. Diets high in fiber have been suggested to increase production of equol,[30,31] and dietary factors, including fiber, may alter intestinal transit time or change the composition of intestinal microflora to increase deconjugation of isoflavones for absorption and metabolism.[32] Cellulose, an insoluble fiber, is the fiber source in AIN-93G diets, and in our experimental diets, the amount of cellulose was adjusted to account for the fiber provided by tomato powder and/or soy germ in those respective diets. Although diets in this study were adjusted to contain equal amounts of fiber, tomato powder containing diets would have had more soluble, fermentable fiber that could support the intestinal microflora that metabolize isoflavones. The positive interaction between tomato and soy on isoflavone bioavailability may also depend on the type of tomato or soy product consumed. Men with recurring prostate cancer that consumed a soy protein supplement in combination with a tomato-rich diet for 4 weeks had significantly lower urinary isoflavones than when they consumed just the soy protein supplement.[8] Men on the tomato-rich diet primarily consumed tomato juice (~11 servings/week), which is low in fiber, rather than raw tomatoes (~4 servings/week),[8] which may explain why consumption of tomato powder, a source of fiber, may have increased urinary isoflavones in rats but not in men consuming tomato juice.

Increased bioavailability of isoflavones in the long-term TP+SG group could be partially explained by the activity of tomato polyphenols (quercetin and kaempferol). In mice, the bioavailability of the isoflavone biochanin A was increased by co-administration of the polyphenols quercetin and epigallocatechin-3-gallate as indicated by increased plasma concentration and urinary excretion of biochanin A.[33] Investigation into the possible mechanism suggested that polyphenols may inhibit efflux transporter mediated elimination of biochanin A and increase the amount of the more bioavailable aglycone by competing for glucuronidation/sulfation in the intestine.[33] Our results suggest that long-term consumption of tomato powder polyphenols may have had a similar interaction with soy isoflavones, resulting in increased urinary isoflavones.

Utilization of a combination of foods and bioactives is gaining interest in the prevention of chronic disease. Whole foods are a complex matrix composed of a mixture of nutrients and bioactives, and it should not be assumed that all combinations of functional foods will be more effective. Results from this study emphasize the importance of preclinical trials to ensure bioavailability of the bioactives of interest and potential interactions between functional foods that could alter the efficacy or safety of dietary interventions.

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Funding Sources

This research was funded in part by the USDA National Needs Training Grant (2005-03750) and the University of Illinois Division of Nutritional Sciences Margin of Excellence Research Program.

ACKNOWLEDGMENT

We thank FutureCeuticals for donating the tomato powder and Frutarom for donation of the soy germ powder and isoflavone analysis of the diets. Undergraduate research assistants Caroline Bauer, Jeremy Park, and Eliza Chan assisted in animal husbandry, carotenoid extractions, and RT-PCR analysis, respectively. Sonya Volker for assistance with enzyme activity assays.

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