Combinations of Tomato and Broccoli Enhance Antitumor Activity in Dunning R3327-H Prostate Adenocarcinomas

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Abstract

The consumption of diets containing 5 to 10 servings of fruits and vegetables daily is the foundation of public health recommendations for cancer prevention, yet this concept has not been tested in experimental models of prostate cancer. We evaluated combinations of tomato and broccoli in the Dunning R3327-H prostate adenocarcinoma model. Male Copenhagen rats (n = 206) were fed diets containing 10% tomato, 10% broccoli, 5% tomato plus 5% broccoli (5:5 combination), 10% tomato plus 10% broccoli (10:10 combination) powders, or lycopene (23 or 224 nmol/g diet) for ∼22 weeks starting 1 month prior to receiving s.c. tumor implants. We compared the effects of diet to surgical castration (2 weeks before termination) or finasteride (5 mg/kg body weight orally, 6 d/wk). Castration reduced prostate weights, tumor areas, and tumor weight (62%, $P < 0.001$), whereas finasteride reduced prostate weights ($P < 0.0001$), but had no effect on tumor area or weight. Lycopene at 23 or 224 nmol/g of the diet insignificantly reduced tumor weights by 7% or 18%, respectively, whereas tomato reduced tumor weight by 34% ($P < 0.05$). Broccoli decreased tumor weights by 42% ($P < 0.01$) whereas the 10:10 combination caused a 52% decrease ($P < 0.001$). Tumor growth reductions were associated with reduced proliferation and increased apoptosis, as quantified by proliferating cell nuclear antigen immunohistochemistry and the ApopTag assay. The combination of tomato and broccoli was more effective at slowing tumor growth than either tomato or broccoli alone and supports the public health recommendations to increase the intake of a variety of plant components. [Cancer Res 2007;67(2):836–43]

Introduction

The roles of fruit and vegetable intake as a modifiable risk factor for cancer has been the subject of extensive epidemiologic investigation (1–4) and led to public health recommendations by the U.S. Department of Agriculture (5), American Cancer Society (6), and the American Institute for Cancer Research (2). Indeed, it is recommended to consume between two cups of fruit and two and a half cups of vegetables per day and to choose a variety of colored fruits and vegetables to lower the risk of cancer. When these nutritional guidelines are followed, there is a significant effect on reducing cancer incidence and mortality (7).

It has been estimated that in 2006, prostate cancer will account for 33% of new cancer cases and 9% of cancer deaths in U.S. men; affecting ∼261,000 men (6). Dietary modifications which delay prostate cancer growth could increase longevity, reduce the need for superfluous medical treatments, lessen morbidity and mortality, and improve overall quality of life. Cancer biologists have typically approached nutrition and cancer risk with a "reductionist" approach. The majority of cell biology and experimental carcinogenesis studies have examined specific chemical components derived from fruits and vegetables. β-Carotene serves as a prototypical example of this approach which led to negative results in two phase III randomized cancer prevention trials, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (8) and the Carotene and Retinol Efficacy Trial (9). The alternative approach, focusing on whole foods, has been less vigorously pursued, due to the chemical complexity of specific foods, variability in composition, difficulty in characterizing mechanism(s) of action, and perceived obstacles to completing randomized clinical trials with whole foods.

Findings from large prospective epidemiologic trials (10–12) first raised awareness that tomatoes, particularly processed tomato products, consumed at a rate of approximately five to seven servings per week, were associated with a 30% to 40% reduction in prostate cancer risk. A recent meta-analysis revealed that compared with those who consumed raw tomatoes infrequently, the relative risk (RR) for prostate cancer in the highest quartile of intake was 0.89 [95% confidence interval (CI), 0.80–1.00], and for those consuming cooked tomato products, the RR was 0.81 (95% CI, 0.71–0.92; ref. 13). As a surrogate indicator of consumption of tomato products, studies evaluating blood concentration of the primary tomato carotenoid, lycopene, have provided supportive evidence of an inverse relationship between serum lycopene and prostate cancer occurrence (14). In addition, the human prostate contains tomato carotenoids (15) and intervention studies show that biomarkers related to prostate carcinogenesis may be altered by dietary intervention with tomato products (16, 17).

A large study using the *N*-methyl-*N*-nitrosourea androgen–induced model of prostate carcinogenesis showed that the anticarcinogenic effects of freeze-dried tomato powder could not be accounted for by lycopene alone (18). Feeding tomato powder resulted in significantly longer survival due to fewer prostate cancer deaths than did feeding control diet (hazard ratio, 0.74; 95% CI, 0.59–0.93; P = 0.009). The lycopene-containing diet caused a nonsignificant reduction in survival from prostate cancer (hazard ratio, 0.91; 95% CI, 0.61–1.35; P = 0.630). Although the vast majority of cell and molecular mechanistic studies have focused on lycopene, a variety of other phytochemicals with anticancer activity are found in tomatoes (19).

A relationship between cruciferous vegetables such as broccoli and prostate cancer emerged more recently from epidemiologic

studies, and a recent review of the literature suggested that the majority of studies supported a protective benefit of consuming cruciferous vegetables (20). Recently, the HPFS cohort showed an inverse association between crucifer intakes and organ-confined prostate cancer (RR, 0.88; 95% CI, 0.74–1.05, *P* for trend = 0.06; ref. 21). Furthermore, in men under the age of 65, there was an inverse relationship between cruciferous vegetable intake and risk of prostate cancer (RR, 0.81; 95% CI, 0.64–1.02, *P* for trend = 0.02), and organ-confined prostate cancer (RR, 0.72; 95% CI, 0.54–0.97, *P* for trend = 0.007). Few studies have examined whole cruciferous vegetables or their components in prostate carcinogenesis using rodent models. Sulforaphane, a putative anticarcinogen in broccoli, was provided orally to mice bearing androgen-insensitive human PC-3 xenografts, and resulted in tumor volumes of 207 ± 35 and 90 ± 22 mm^3 for the control and sulforaphane groups, respectively (22). Additionally, tumor weights in the sulforaphane-treated mice were one-fourth those of the control tumors (*P* < 0.05).

Androgens are essential for the initiation and progression of prostate cancer. In order to provide a relative comparison of the efficacy of tomato and/or broccoli powders as antitumor agents, we have compared dietary interventions with disruption of the androgen-hormonal axis, including finasteride or castration as additional controls. Finasteride acts by competitively inhibiting 5-α-reductase type II, the enzyme responsible for the conversion of testosterone to the more potent androgen, dihydroxytestosterone (23). The Prostate Cancer Prevention Trial examined the chemopreventive effects of finasteride (5 mg/d for 7 years) and found a 24.8% decrease in the prevalence of prostate cancer, yet there was an increase in tumors considered high grade to 6.4% versus 5.1% in the placebo-treated group (*P* < 0.001; ref. 24). Castration has been a successful intervention for prostate cancer therapy for >60 years (25).

Our laboratory is actively investigating the hypothesis that combinations of food-based cancer prevention strategies will be a highly effective strategy for the reduction of prostate carcinogenesis. Remarkably, almost no quantitative information regarding the efficacy or safety of combinations of foods has been published using precisely defined diets and rodent models of prostate cancer. We have chosen to focus our experimental efforts on tomatoes and broccoli, two foods frequently cited to protect men from prostate carcinogenesis (14, 20). This report focuses on a well-characterized androgen-dependent Dunning R3327-H transplantable prostate cancer model (26), with diets supplemented with modest proportions of freeze-dried tomato and/or broccoli powders. Tumor growth was the primary outcome and complemented by a series of selected serum and histopathologic biomarkers as secondary outcomes. Specifically, proliferating cell nuclear antigen (PCNA) as an indicator of proliferation and the ApopTag assay to quantify rates of apoptosis. To our knowledge, we are the first to examine the effects of a whole crucifer, broccoli, in an animal model of prostate cancer, and the first to test the anticancer potential of the combination of tomato and broccoli powders.

Materials and Methods

Animals. Two hundred and six male Copenhagen rats (Cop 2331; Harlan, Indianapolis, IN) were obtained at ∼ 4 weeks of age and individually housed in wire-bottomed cages. Rats were monitored daily and weighed on a weekly basis. Food efficiency was calculated using the equation (food efficiency = g body weight gained/g of diet consumed). The University of Illinois Laboratory Animal Care Advisory Committee approved all animal procedures.

Experimental diets. AIN93G-based diets were fed *ad libitum* beginning 1 month prior to tumor implantations, and were continued throughout the study. Diets were balanced for protein, fat, energy, and fiber, and stored at 4°C in the dark (Table 1). Test ingredients included 5% water-dispersible lycopene beadlets (DSM, Basel, Switzerland) and whole tomato and broccoli powders which are commercially produced and freeze-dried for human consumption (Gilroy Foods, Gilroy, CA). Experimental diets included: 10% tomato powder, 10% broccoli powder, 10% tomato with 10% broccoli (10:10 combination), 5% tomato with 5% broccoli (5:5 combination), and two levels of AIN93G control diet with lycopene supplementation at either 23 or 224 nmol lycopene/g diet.

Dunning R3327-H prostate tumor implantations. Dunning R3327-H tumor tissue coated with Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA) was implanted s.c. in each rear flank of donor rats and allowed to grow for 18 weeks to ∼ 2 cm diameter as a source of tumor for the two experimental trials. Viable, nonnecrotic tumor tissue was removed from donor animals, minced, pressed through a 20-mesh sieve, and suspended in Matrigel to obtain a final concentration of 100 mg tumor/mL, and 0.2 mL (20 mg of tumor) was implanted via a s.c. injection into each rear flank of 206 rats. Two trials of the study were done, with three cohorts of rats in each trial, and every cohort contained three to five control rats. Tumor areas were measured weekly using calipers. Two diameter measurements were taken, 90 degrees apart, and the area was calculated [area (mm^2) = radius (mm)^2 × π]. The trails were terminated ∼ 18 weeks after tumor implantations.

Androgen ablation and serum androgen analysis. For chemical androgen ablation in the first trial, rats were fed AIN93G control diet and were oral gavaged 5 mg/kg body weight finasteride (Proscar, Merck and Co., Inc., Whitehouse Station, NJ) 6 d/wk beginning 3 weeks after tumor implantations. The oral dose of finasteride was prepared using a mortar and pestle to crush the Proscar tablets which were then dissolved in deionized water. For surgical castration in the second trial, both testes were removed from a group of AIN93G control–fed rats 16 weeks after tumor implantations. Serum testosterone and dihydrotestosterone concentrations were measured by coated tube RIA using DSL-400 and DSL-9600 kits, respectively (Diagnostic Systems Laboratories, Inc., Webster, TX).

Tissue histology and immunohistochemistry. A section of each Dunning R3327-H tumor was preserved in 10% formalin for 24 to 48 h before being moved to 70% ethanol. Formalin-fixed tissues were shipped to Ohio State University's Comprehensive Cancer Center for further processing. Samples were embedded in paraffin using a Tissue Embedding System (Shandon Histocentre 2, Shandon, Pittsburgh, PA), sectioned, and transferred to Superfrost plus Slides (Fisher Scientific, Pittsburgh, PA).

PCNA staining was done and evaluated according to lab protocols previously published (27). Images without necrosis or artifacts were captured by a digital camera (Spot RT, Diagnostic Instrument, Inc., Sterling Heights, MI) at 400× magnification using bright-field microscopy (Nikon ECLIPSE E 800, Tokyo, Japan). The number of nuclei stained positive for PCNA were counted and used to calculate the proliferative index percentage = (PCNA positive–stained nuclei / total nuclei counted) × 100 for each Dunning R3327-H tumor.

The ApopTag Kit (Chemicon International, Inc., Temecula, CA) was used as in previous experiments in our laboratories (27, 28). Two representative areas lacking necrosis and artifacts were selected from each tumor and apoptotic nuclei were counted at 400× magnification.

Lycopene quantification. Lycopene extractions were done using techniques previously described by our laboratory (29). In short, diet, serum, and tissue samples were extracted using hexane and run on a C30 high-performance liquid chromatography (HPLC) column with gradient mobile phases consisting of methanol, methyl-tert butyl ether, and ammonium acetate with the UV detector set at a wavelength of 450 nm.

Glucosinolate analysis. Samples of broccoli powder were heated in boiling water, centrifuged, and the glucosinolates desulfated and separated by HPLC using a Lichosphere RP-18 column, a linear gradient of 0% to 20% acetonitrile in water, and detection at 229 nm wavelength, as previously published (30).

Statistics. Data were compared among treatments by two-tailed ANOVA, using SAS Statistical Software (version 8.1; SAS Institute, Cary, NC), and values were considered different from controls at *P* < 0.05 using Student's *t* tests.

Results

Animals. Diets were well received and consumed by all groups. Rats gained weight as expected throughout the study. There were no significant differences in food efficiency among treatment groups (data not shown). Whereas no dietary treatments affected prostate weight, finasteride and surgical castration reduced prostate weights to 39% and 44% of controls, respectively (Table 2). The variations seen in serum testosterone (Table 2) were within physiologic reference ranges (31), and no differences were found in serum dihydroxytestosterone (data not shown).

Tumor areas. Dunning R3327-H tumor incidence was ∼92% for all implantation sites, regardless of trial, cohort, or treatment. Tumor areas for each of the two trials can be seen in Fig. 1A and C. Tumor areas across all groups were larger in the first trial (Fig. 1A) than in the second trial (Fig. 1C), yet equivalent patterns were observed in response to the dietary treatments in both trials. Dietary treatment with the 10:10 combination resulted in tumor areas different than control rats starting 12 weeks after tumor implantation ($P_{week\ 12} = 0.01$) and these differences continued throughout the study ($P_{week\ 18} = 0.001$). The 5:5 combination reduced tumor areas starting at week 13 ($P_{week\ 13} = 0.01$; $P_{week\ 18} = 0.001$), tomato by 12 weeks ($P_{week\ 12} = 0.05$; $P_{week\ 18} = 0.001$), broccoli by week 13 ($P_{week\ 13} = 0.01$; $P_{week\ 18} = 0.001$), and the two lycopene-supplemented groups reduced tumor areas by week 15 ($P_{23\ nmol/g\ diet} = 0.05$; $P_{224\ nmol/g\ diet} = 0.001$). Castration in trial 2 had a dramatic and immediate effect on tumor area, decreasing tumor areas by >56% in 2 weeks ($P = 0.001$). The other method of androgen deprivation, finasteride, resulted in no differences in tumor areas compared with the control.

Tumor weights. As seen with tumor areas, the tumor weights were larger in the first trial (Fig. 1B) than in the second trial (Fig. 1D); nevertheless, similar outcomes due to dietary interventions occurred in both trials. Therefore, tumor weight data for both trials were normalized and expressed as a percentage of each of the six cohort's control tumor weights; with control tumor weights expressed as 100%. Table 2 shows merged tumor weight results expressed as a percentage of controls.

Rats consuming diets containing broccoli powder ($P < 0.01$), the 10:10 combination ($P < 0.001$), or following castration ($P < 0.001$), had reduced tumor weights compared with tumor weights in control animals (Table 2). The 224 nmol lycopene/g diet was not as effective in reducing tumor weights ($P = 0.24$) as the tomato powder diet ($P < 0.05$). The 5:5 combination resulted in a trend for reduction in tumor weights ($P = 0.055$). Neither the 23 nmol lycopene/g diet nor the finasteride treatment affected tumor weights compared with controls.

Lycopene concentrations. Serum, liver, prostate, and Dunning R3327-H tumor lycopene concentrations can be seen in Table 3. No lycopene was detected in the diet, serum, or tissues of control, broccoli-fed, finasteride, or castrated rats. Interestingly, although the 23 nmol lycopene/g diet provided twice as much lycopene as the 10% tomato diet and almost four times more than the 5:5 combination diet, the tissue levels of lycopene in rats fed the 23 nmol lycopene/g diet were much lower. The bioavailability of lycopene from the lycopene beadlets

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Table 1. AIN 93G–based diet formulations

	Control	23 nmol lycopene/g diet*	224 nmol lycopene/g diet[†]	Tomato[‡]	Broccoli	5:5 Combination[§]	10:10 Combination[‖]
			g/100 g total diet				
Cornstarch	39.7	39.65	39.2	34.8	37.0	35.8	32
Casein	20.0	20.0	20.0	18.6	16.9	17.8	15.5
Maltodextrin	10.2	10.2	10.2	8.8	9.6	9.2	8.2
Sucrose	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Fiber[¶]	5.0	5.0	5.0	3.2	1.8	2.5	0.1
Mineral mix**	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix[††]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
L-Cystine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline bitartrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Soybean oil	10.0	10.0	10.0	9.5	9.6	9.6	9.1
Lycopene beadlet		0.05	0.50				
Tomato powder				10.0		5.0	10.0
Broccoli powder[‡‡]					10.0	5.0	10.0

*As measured by HPLC, this diet contains 23 nmol of lycopene per gram of diet (0.05 g lycopene beadlets per 100 g diet).
†As measured by HPLC, this diet contains 224 nmol of lycopene per gram of diet (0.5 g lycopene beadlets per 100 g diet).
‡As measured by HPLC, this diet contains 13 nmol of lycopene per gram of diet (10% tomato powder diet).
§As measured by HPLC, this diet contains 6 nmol of lycopene per gram of diet (5% tomato and 5% broccoli diet).
‖As measured by HPLC, this diet contains 11 nmol of lycopene per gram of diet (10% tomato and 10% broccoli diet).
¶Fiber source was non-nutritive cellulose.
**AIN93M-MX formulation.
††AIN93-VX formulation.
‡‡Each gram of broccoli powder contains 1.6 μmol of glucoraphanin, 5.9 μmol of glucobrassicin, 3.9 μmol of gluconasturtiin, and 2.1 μmol of neoglucobrassicin.

Table 2. Dunning R3327-H tumor weights, prostate weights, and serum testosterone

Treatment group	Group size (n = rats)	Prostate weights (% body weight)*	Serum testosterone (ng/mL)*, [†]	Tumor weight (% control)*	Tumor weight (P values) [‡]
Control	26	0.23 ± 0.01	1.7 ± 0.1[AB]	100 ± 10	—
Finasteride	12	0.09 ± 0.01[§]	1.9 ± 0.2[AB]	86 ± 17	—
Castration	15	0.10 ± 0.01[§]	Not detectable	38 ± 6	0.001
23 nmol lycopene/g diet	23	0.27 ± 0.01	1.4 ± 0.1[B]	93 ± 15	—
224 nmol lycopene/g diet	27	0.25 ± 0.01	1.9 ± 0.1[AB]	82 ± 10	0.24
10% Tomato	27	0.25 ± 0.01	1.7 ± 0.1[AB]	67 ± 7	0.05
10% Broccoli	26	0.25 ± 0.01	1.6 ± 0.1[AB]	58 ± 7	0.01
5:5 Combination	24	0.27 ± 0.01	2.0 ± 0.1[A]	70 ± 8	0.055
10:10 Combination	26	0.26 ± 0.01	1.9 ± 0.1[AB]	48 ± 6	0.001

*Data are mean ± SE for both study trials.
[†] Values with different letters are statistically different ($P = 0.01$).
[‡] P values indicate difference from control tumor weights.
[§] Statistically different from all other treatments ($P < 0.0001$).

versus the tomato powder is being investigated further in our laboratory.

Glucosinolate analysis. HPLC analysis revealed the presence of four major glucosinolates; glucoraphanin, glucobrassicin, gluconasturtiin, and neoglucobrassicin; the concentrations per gram of broccoli powder are shown in the footnote of Table 1.

PCNA staining. Nuclei from 20 tumors per group were counted in duplicate for PCNA staining. Similar trends were seen between the PCNA and ApopTag results in tumors from each trial, hence the data have been combined. Proliferative index percentages can be seen in Fig. 2*A* and samples of high and low PCNA staining can be seen in Fig. 3*A* and *B*. Lycopene supplementation at a level of 224 nmol/g diet ($P = 0.05$), castration ($P = 0.05$), the 5:5 combination diet ($P = 0.05$), tomato ($P = 0.001$), the 10:10 combination diet ($P = 0.001$), and broccoli ($P = 0.0001$) all had reduced proliferative index percentages compared with control tumors.
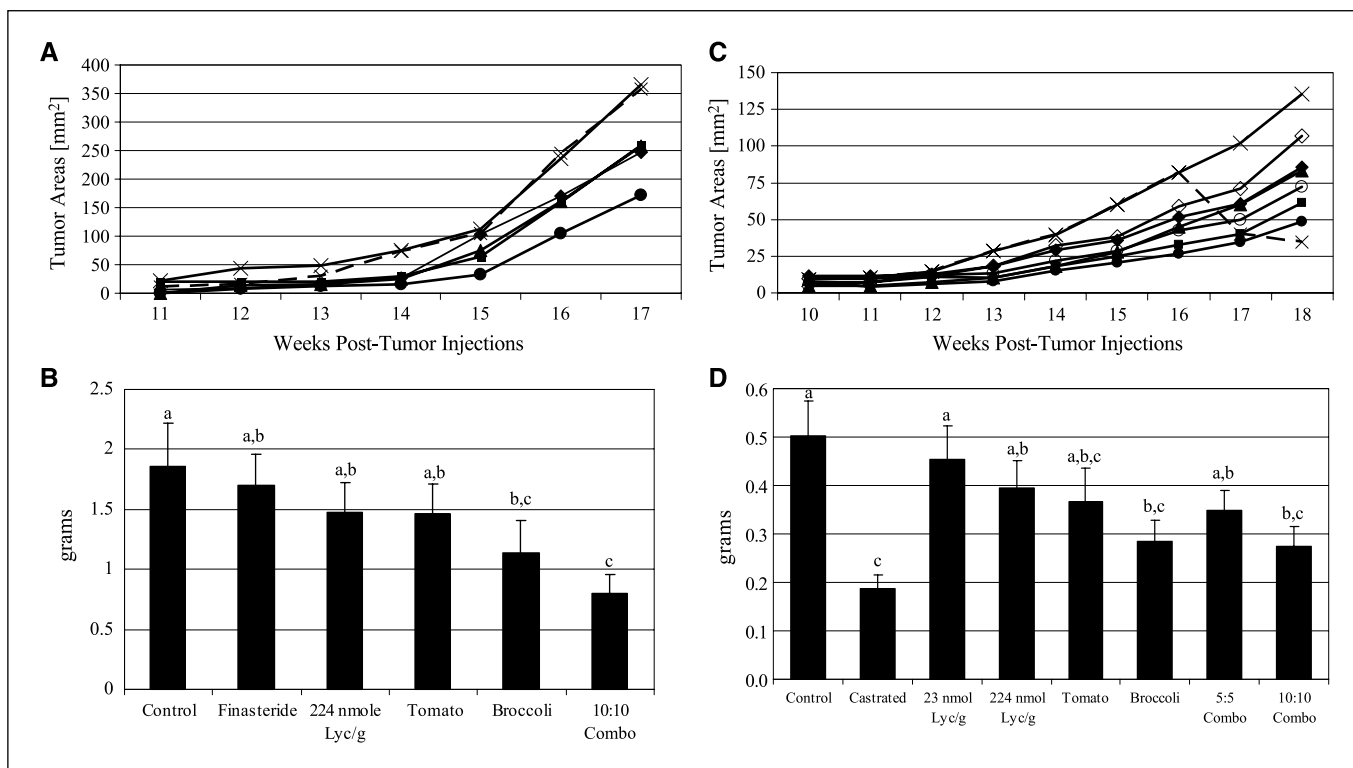

Figure 1. *A,* trial no. 1 tumor areas; *C,* trial no. 2 tumor areas; control (—×—), tomato (—▲—), broccoli (—■—), 224 nmol lycopene/g diet (—◆—), 23 nmol lycopene/g diet (—◇—), 5:5 combination (—○—), 10:10 combination (—●—); *broken line with* (—×—), finasteride in trial no. 1, castration in trial no. 2. *B,* trial no. 1 tumor weights ± SE; *D,* trial no. 2 tumor weights ± SE; bars with different letters are significantly different ($P = 0.05$).

Table 3. Serum and tissue lycopene concentrations

Treatment group	Tissue lycopene concentration (nmol lycopene/g)*			
	Serum	Liver	Prostate [†]	Dunning tumor
Control	ND	ND	ND	ND
Finasteride	ND	ND	ND	ND
Castrated	ND	ND	ND	ND
23 nmol lycopene/g diet	252 ± 28	58 ± 4	0.1 ± 0.01	0.13 ± 0.01
224 nmol lycopene/g diet	884 ± 80	527 ± 40	0.9 ± 0.08	0.58 ± 0.03
10% Tomato	511 ± 41	189 ± 15	0.5 ± 0.06	0.40 ± 0.03
10% Broccoli	ND	ND	ND	ND
5:5 Combination	439 ± 48	123 ± 14	0.3 ± 0.01	0.26 ± 0.03
10:10 Combination	538 ± 39	160 ± 9	0.4 ± 0.02	0.42 ± 0.03

Abbreviation: ND, lycopene was not detected.
*Data are mean $\pm$ SE for both study trials.
[†]Dorsolateral + anterior lobes of the rat prostate.

Apoptosis rates. An average of 27 tumors per group were counted and evaluated for apoptosis rates. Although neither lycopene group altered apoptotic rates in the Dunning R3327-H tumors, the tomato group ($P = 0.01$) increased apoptosis compared with control tumors (Fig. 2B). Castration ($P = 0.001$) and feeding either 10% broccoli alone ($P < 0.0001$), or in combination with 10% tomato ($P < 0.0001$), also increased apoptosis rates in the Dunning R3327-H tumors. Examples of ApopTag staining can be seen in Fig. 3C and D.

Discussion

This Dunning R3327-H *in vivo* prostate cancer study, which employed carefully defined dietary interventions, reinforces the findings of epidemiologic studies demonstrating protective associations for diets rich in tomatoes and broccoli against prostate cancer. In addition, these findings provide support for future human prevention trials based on dietary interventions. Although both tomato and broccoli powder show anticancer activity as individual agents, our studies suggest that additional gains are achieved through the combination of these foods. It is implausible that a single mechanism will account for the anticancer effects resulting from the myriad of bioactive phytochemicals in broccoli and tomatoes. However, biomarkers such as enhanced apoptosis and reduced proliferation correlated with the anticancer activity seen with the consumption of tomato and broccoli, and may provide leads into the mechanisms influenced by dietary changes. Furthermore, these findings support the public health approach to diet and cancer prevention exemplified by the Dietary Guidelines for Americans, 5aday.gov and Mypyramid.gov supported by the American Institute for Cancer Research, Centers for Disease Control, U.S. Department of Agriculture, and NIH/National Cancer Institute. Each of these agencies support a recommendation to regularly consume between 5 and 10 servings of a variety of colored fruits and vegetables in order to reduce cancer risk (5).

Many investigators and purveyors of supplements for cancer prevention consider lycopene to account for the anticancer properties of tomato products. In our study, we provided lycopene at 23 nmol/g diet, which provided lycopene at a concentration similar to the 10% tomato diet, and a 10-fold greater dose at 224 nmol/g diet. Interestingly, neither dose of lycopene was able to

significantly reduce tumorigenesis (Tables 1 and 3), whereas 10% whole tomato powder significantly reduced tumor weights ($P < 0.05$). Our work (18) and other's (32–34) suggest that lycopene alone may have some antiprostate cancer activity, but the whole tomato and its array of phytochemicals clearly shows anticancer potential that exceeds the pure carotenoid.

Lycopene is the most efficient of all common dietary carotenoids tested *in vitro* at scavenging singlet oxygen and reactive oxygen species, and it has been suggested that this may be one of its anticancer properties (35). We should consider that this
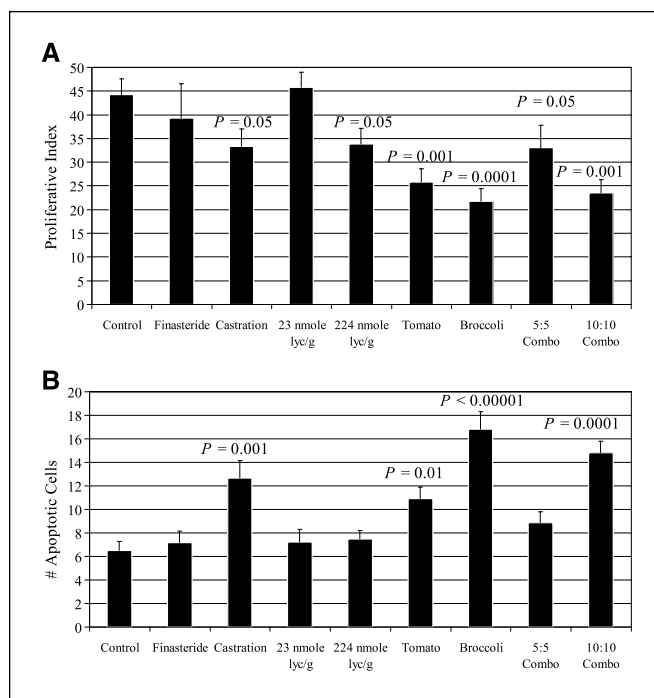

Figure 2. Proliferation (*A*) and apoptosis (*B*) rates of Dunning R3327-H tumors. Numbers above columns are *P* values which indicate differences from the control group.
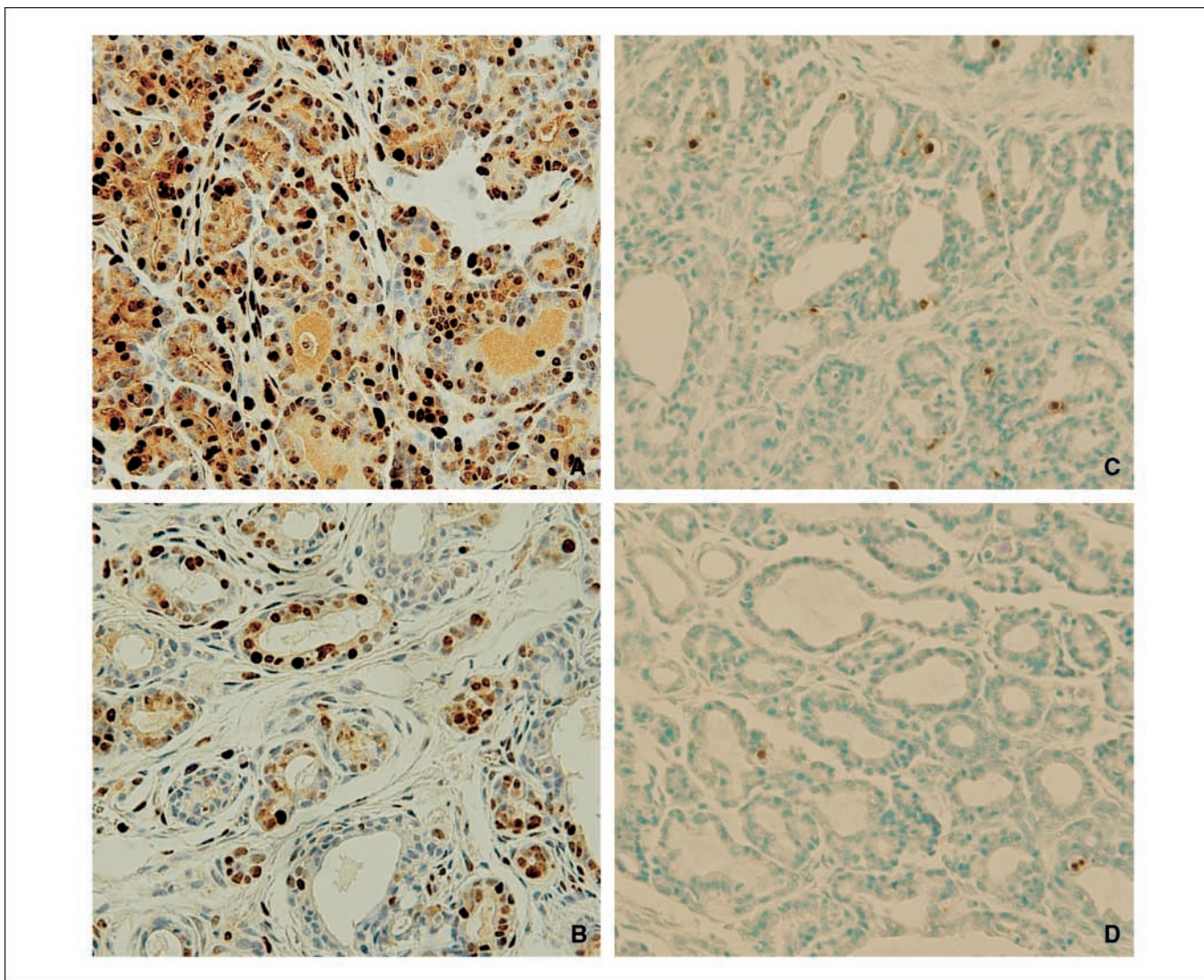
Figure 3. Examples of Dunning R3327-H tumor immunohistochemistry, brown 3,3′-diaminobenzidine–stained nuclei indicative of either a cell undergoing proliferation in the case of (*A*) and (*B*), or apoptosis in the case of (*C*) and (*D*). *A,* high PCNA staining; *B,* low PCNA staining; *C,* high ApopTag staining; *D,* low ApopTag staining (original magnification, ×400).

mechanism of action seems more relevant to the long-term carcinogenesis process in humans, where oxidative stress is currently implicated, than in a transplantable model such as that used in this study. Other proposed anticancer mechanisms of action for lycopene include decreased DNA strand breaks, antiandrogen, and anti–growth factor effects (36). Tomatoes contain many bioactive components including vitamins C, K, E, as well as fiber, folate, and polyphenols such as quercetin (USDA NDB No: 11529; refs. 19, 37). HPLC analysis of the tomato powder diet also shows significant amounts of other carotenoids, including phytoene and phytofluene, which should also be considered for potential anticancer activity.

To our knowledge, this is the first study using broccoli to examine prostate tumorigenesis in a rodent model. Broccoli powder inhibited tumor growth by ~42% compared with rats on the control diet (*P* = 0.01). Broccoli contains a vast array of potentially bioactive components, including glucosinolates that can be hydrolyzed to metabolites with known anticarcinogenic activity,

particularly sulforaphane and indole-3-carbinol (38, 39). Broccoli also contains numerous bioactive components, including folate, potassium, selenium, vitamins A, C, E, carotenoids, and polyphenols (USDA NDB no: 11090; ref. 19). Various broccoli phytochemicals have been hypothesized to affect prostate carcinogenesis by multiple mechanisms including the suppression of proliferation and induction of apoptosis, decreased expression of the androgen receptor, induction of detoxification enzymes, inhibition of CYP-dependent activation of carcinogens, inhibition of nuclear factor κB transcriptional activity, and inhibition of the cell cycle (22, 40–42). A role for broccoli in prostate cancer prevention is now supported by epidemiologic, rodent, and cell culture studies.

This study supports the concept that a change in dietary pattern may have a much greater anticancer benefit than can be derived from changes in a single food item or a single component of a food provided as a supplement. The combination of 10% tomato with 10% broccoli powders slowed Dunning R3327-H adenocarcinoma growth to <50% of control tumor growth (*P* = 0.001). This effect is presumed

to be due to an increase in the variety of phytochemicals present in the 10:10 combination diet which may target numerous different pathways associated with tumorigenesis. This seems to be the first *in vivo* rodent study to show that a dietary change combining two different fruits or vegetables may show superior benefits on prostate cancer growth. This food-based concept, although not shown in prostate carcinogenesis, has been examined in other tumor types. For example, the potential interaction between tomato and garlic components in the hamster buccal pouch carcinogenesis model was assessed (43). After treatment with 7,12-dimethylbenz(*a*) anthracene the mean tumor burden (mm^3) was 945 ± 81.8, 662 ± 65.2, 589 ± 61.2, and 198 ± 27.0 for the control, tomato, garlic, and tomato plus garlic groups ($P < 0.01$), respectively. Thus, it certainly is reasonable to continue with reductionist nutrition and cancer research in which specific plant-based phytochemicals are isolated and considered for chemopreventive activity, yet our work strongly supports the importance of considering more complex dietary changes involving whole foods containing multiple biologically active compounds (44, 45).

Our approach to selecting tumor biomarkers for evaluation was to examine proliferation (46) and apoptosis (28), both of which represent the culmination of a variety of signaling cascades potentially influenced by tomato and broccoli phytochemicals. In the current study, 224 nmol lycopene/g diet ($P = 0.05$), castration ($P = 0.05$), 5:5 combination ($P = 0.05$), tomato ($P = 0.001$), 10:10 combination ($P = 0.001$), and broccoli ($P = 0.0001$) were all associated with suppressed proliferation rates, roughly in parallel with tumor growth. Proliferative index using PCNA has been proposed as a prognostic factor in human prostate cancer, and has been associated with a higher risk of aggressive disease (47). Sulforaphane decreased PCNA in F3II mammary carcinoma implants in BALB/C mice (48), but no reports are available on the effects of broccoli on PCNA in prostate cancer.

One mechanism that may be relevant to cancer prevention by dietary phytochemicals is regarding their ability to alter the apoptotic threshold (28). Broccoli components have shown enhanced apoptosis via an increase in the Bax/Bcl-2 ratio and p21, activation of caspase 3 and caspase 8, and cleavage or inactivation of poly(ADP-ribose)polymerase (22, 49, 50). *In vitro*, lycopene has been shown to inhibit the growth of human androgen-independent PC cells and rat Dunning AT6.3 cells, as well as *in vivo* by increasing the apoptotic rate in the human prostate cells (17, 28, 51). Tomato polyphenols also enhanced the sensitivity to apoptosis in the Dunning AT6.3 cell line via inhibition of growth factor–induced AKT-signaling (28). Interestingly, in the Dunning R3327-H prostate cancer model, neither lycopene-fed group showed changes in apoptotic rates in tumors, yet tomato consumption resulted in increased apoptosis ($P = 0.01$; Fig. 2). Castration ($P = 0.001$) and feeding either 10% broccoli alone ($P < 0.0001$) or in combination with 10% tomato ($P < 0.0001$) were also successful in increasing the apoptosis rates in Dunning R3327-H tumors.

We included treatment groups who received finasteride or underwent castration in order to have a direct quantitative comparison with dietary interventions. In the present study, finasteride, which has chemopreventive properties in humans (24), did not significantly alter Dunning R3327-H tumor growth, serum testosterone, or serum dihydroxytestosterone levels. Yet, the normal prostate (Table 2) and seminal vesicle weights (data not shown) were significantly decreased ($P < 0.0001$), thus, confirming the expected biological activity of the drug. Clearly, the Dunning R3327-H androgen-dependent prostate tumor is not sensitive to 5-α-reductase II inhibition. Thus, it is of interest that tomato or broccoli exposure, alone or in combination, was more effective than finasteride which has shown a 24% reduction in prostate tumor detection in a 7-year human study (24). A recently published study found similar results whereby finasteride, provided as high as 72 mg/kg body weight, reduced prostate weight but did not inhibit Dunning R3327-H prostate tumor weights (52). In contrast, castration dramatically reduced tumor growth and induced prostate atrophy. The effects of castration on tumor histology, PCNA, and apoptosis were similar to those of dietary intervention, although we cannot, at this time, conclude that the mechanisms are similar as many different pathways may interact to achieve these changes in biomarker expression.

In conclusion, this Dunning R3327-H prostate adenocarcinoma study shows that intake of whole foods, such as broccoli and tomatoes, could significantly affect prostate cancer tumorigenesis and the combination of whole foods may be superior in slowing cancer growth. Diet-induced changes in tumor growth occur in parallel with changes in tumor biomarkers such as proliferation and apoptosis which are associated with improved clinical outcomes in humans. We propose that studies using a reductionist philosophy to identify active components of whole foods, such as lycopene or sulforaphane, should continue, but with additional investments in research focusing on changes in dietary pattern incorporating whole foods which are safe and potentially more effective. Finally, our work provides direct *in vivo* experimental data to support the current dietary recommendations for Americans, which urges regular consumption of a variety of colored vegetables to reduce the risk of chronic diseases such as prostate cancer.

Acknowledgments

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