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# A 10% Tomato Diet Selectively Reduces Radiation-Induced Damage in TRAMP Mice

Joe L Rowles, III,[1] Matthew A Wallig,[1,2] Kimberly A Selting,[3,4] Timothy M Fan,[3,4] Rita J Miller,[5] William D O'Brien, Jr,[1,5] and John W Erdman, Jr[1,4,6]

[1]Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [2]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [3]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [4]Cancer Center at Illinois, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [5]Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA; and [6]Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, USA

## ABSTRACT

**Background:** Tomatoes contain carotenoids that have the potential to alter the effects of external beam radiation therapy (EBRT).

**Objectives:** We hypothesized that dietary lyophilized tomato paste (TP) would reduce apoptosis within carotenoid-containing nonneoplastic tissues in EBRT-treated TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice.

**Methods:** Male TRAMP mice ($n = 73$) were provided an AIN-93G diet or a modified AIN-93G diet containing 10% TP (wt:wt) at 4 wk of age. Prostate tumor growth was monitored by ultrasound. The caudal half of the mouse was irradiated with 7.5 Gy (Rad) or 0 Gy (sham) at 24 wk of age or after the tumor volume exceeded 1000 $mm^3$ with a Cobalt-60 source. Mice were euthanized 24 h postradiation. Carotenoids and $\alpha$-tocopherol were measured by HPLC and compared by a *t* test. Tissues were assessed for radiation-induced changes (hematoxylin and eosin) and apoptosis [cleaved caspase-3 (CC3)] and compared by Kruskal–Wallis test or Freedman–Lane's permutation test.

**Results:** Serum concentrations of lycopene (52% lower), phytoene (26% lower), and $\alpha$-tocopherol (22% lower) were decreased in TP-fed irradiated mice (TP-Rad) compared with TP-fed sham mice ($P < 0.05$). CC3 scores increased within the prostate tumor with radiation treatments ($P < 0.05$), but were not affected by tomato consumption. In nonneoplastic tissues, TP-Rad had a lower percentage of CC3-positive cells within the cranial (67% lower) and caudal (75% lower) duodenum than irradiated mice on the control diet (Rad) ($P < 0.005$). Likewise, CC3 scores within the dorsolateral prostate of TP-Rad trended toward lower scores than for Rad ($P = 0.07$).

**Conclusions:** TP selectively reduces radiation-induced apoptosis in extratumoral tissues without decreasing radiation-induced apoptosis within the prostate tumor in TRAMP mice. Additional studies are needed to confirm and expand upon these findings. *J Nutr* 2021;151:3421–3430.

**Keywords:** tomato, carotenoids, prostate cancer, radiation, TRAMP

## Introduction

Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in Western countries (1). External beam radiation therapy (EBRT) is one of the most common treatment approaches for PCa (2–4). Despite improvements to treatment, ~50%–60% of men who receive EBRT develop biochemical recurrence (5, 6). In clinical practice, the EBRT is limited by potential damage to the surrounding (nonneoplastic) tissues. Acute and chronic toxic effects such as bowel and sexual dysfunction (7) constrain the clinical tolerability of EBRT.

After PCa diagnosis, patients often seek information about food and supplements that may improve their response to therapies, quality of life, and survival. Tomatoes and lycopene are 2 of the most frequently mentioned foods or supplements by social media, lay press, and purveyors of alternative therapy as having a protective effect against PCa development and progression. Tomato and lycopene consumption has been associated with decreased risk of PCa in epidemiological studies (8, 9). Lycopene, in addition to other tomato bioactives, may decrease PCa tumor progression by modifying inflammatory status (10, 11), androgen and growth factor signaling (12), apoptosis (11, 13, 14), and cell cycle progression (11, 13, 14).

Lycopene and other tomato carotenoids are potent antioxidants that may improve natural defenses and scavenge free radicals generated during radiation therapy. Antioxidants may quench singlet molecular oxygen [reactive oxygen species (ROS)], suppress the highly proinflammatory environment after EBRT, and potentially alter apoptosis. Improving antioxidant status in normal tissues is radioprotective and may lead to fewer adverse events owing to decreased oxidative damage from


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radiation therapy (15, 16). Although results suggest that tomato products may provide a benefit to patients who are diagnosed with PCa, the lack of preclinical and clinical data for men undergoing EBRT prevents more definitive trials from occurring and remains a gap in the literature.

The objective of this study was to determine the extent to which tomato feeding decreased apoptosis and cell death within tissues (neoplastic and nonneoplastic) that accumulate carotenoids. We hypothesized that carotenoids would reduce apoptosis within irradiated nonneoplastic tissues. To evaluate this hypothesis, we conducted 2 studies in the TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model. The first study was a pilot study that established the timing of radiation that would be used in the dietary study. The second study evaluated whether lifelong tomato powder consumption decreased radiation-induced damage (apoptosis and inflammation) in prostate tumors and associated surrounding tissues. Tomato or lycopene consumption has not been previously evaluated as a method of reducing radiation-induced damage (inflammation or apoptosis) in prostatic tumors and surrounding organs, to our knowledge.

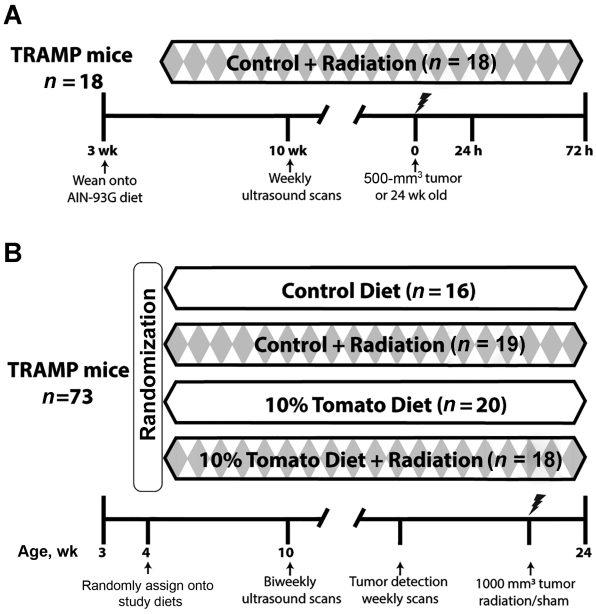


**FIGURE 1** Study design. TRAMP mice were randomly assigned to dietary treatment groups after weaning (4 wk). Prostates were monitored weekly (pilot study; A) or biweekly (diet study; B) for tumor occurrence by ultrasound beginning at 10 wk of age. After tumor detection, mice were scanned weekly by ultrasound until tumors reached a volume of 500 $mm^3$ (pilot study) or 1000 $mm^3$ (diet study). At this volume, the caudal half of the animal was irradiated with 7.5 Gy by a Co-60 source. Mice were euthanized 24 or 72 h after radiation or after a sham treatment. TRAMP, TRansgenic Adenocarcinoma of the Mouse Prostate.

## Methods

### Diets

Tomato paste (TP) (Contadina®) was purchased from a local supermarket in September 2014, July 2015, and April 2016 and lyophilized in a VirTis Freezemobile 12SL/Unitop 600 SL freeze dryer (SP Scientific). Lyophilized TP was ground into a fine powder using a tabletop food processor, transferred to resealable gallon bags (air removed), and kept in the dark at −20°C to preserve the carotenoids and other nutrients in the TP (17, 18).

Two experimental diets were used: a powdered, AIN-93G-based control diet and the same diet modified to contain 10% (wt:wt) lyophilized TP. Proximate analysis was performed on the 100% TP powder and diet formulas were balanced for total energy, carbohydrates, protein, fat, fiber, and moisture. AIN-93G vitamin and mineral mixes were provided to both diets in equal proportions. **Supplemental Table 1** describes the composition of the control and tomato diets. Ingredients were mixed using a commercial mixer (Hobart). New diets were formulated every 2 mo. Carotenoid and α-tocopherol contents of each batch were analyzed by HPLC.

### Mouse breeding, genotyping, and housing

The University of Illinois Laboratory Institutional Animal Care and Use Committee approved all experimental procedures (Protocol 18029). Male C57BL/6-Tg(TRAMP)8247Ng/J (C57BL/6 TRAMP+/−), female C57BL/6J, and female FVB/NJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Breeding and genotyping of these mice were conducted as previously described (18). Males carrying the probasin:SV40-Tag transgene (TRAMP mice) were weaned at 3 wk of age and enrolled into the study via rolling admission. Mice were housed under controlled conditions (12-h light/dark cycle, 22°C, 55% humidity), weighed weekly, and diet was added 3 times/wk.

### Pilot study

TRAMP mice were randomly assigned onto an AIN-93G control diet ($n$ = 18) at 4 wk of age. TP was not evaluated in the pilot study. Beginning at 10 wk of age, weekly in vivo ultrasound imaging was used for longitudinal tumor screening and tumor volume measurement. Ultrasound screening of TRAMP prostates with a Vevo 2100 preclinical ultrasonic imaging platform (VisualSonics, Inc.) has been previously described (18). Serial 2D image slices were used to generate prostatic or tumor volume estimates as previously described (19).

Mice were irradiated with 7.5 Gy γ radiation by a Cobalt-60 source at a dose rate of 0.22 Gy/min (Theratron-780 Isocentric teletherapy, Theratronics®) ($n$ = 12) or 0 Gy [sham treatment (Sham), $n$ = 6] at 24 wk of age if a tumor mass was not detected or after the tumor grew to a specific size (500 $mm^3$). The radiation was collimated to protect the cranial half of the mouse. Inhalation isoflurane was used for general anesthesia. Sham-treated mice underwent the same procedure as irradiated mice (including inhalation anesthesia) but received no radiation. A radiation dose of 7.5 Gy of radiation was delivered to PCa-bearing mice. The radiation dose was calculated using the linear-quadratic model to generate a radiation dose isoeffective to 25% of a human's hypofractionated total dose used for the management of PCa (20–23). Thermoluminescent dosimeters (University of Wisconsin Radiation Calibration Laboratory, Madison, WI) were placed under the caudal and cranial halves of the mouse and were used to confirm the delivered radiation dose (**Supplemental Figure 1**). Mice were euthanized 0 (sham mice), 24, or 72 h postradiation ($n$ = 6/time point). **Figure 1** shows study designs for the pilot and diet studies.

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Supplemental Tables 1–5, Supplemental Figures 1–3, and Supplemental Appendixes 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
Present address for JLR: Department of Chemistry, Washington University in Saint Louis, One Brookings Drive, St Louis, MO 63130, USA.
Address correspondence to JWE (e-mail: jwerdman@illinois.edu).
Abbreviations used: CC3, cleaved caspase-3; CRP, C-reactive protein; EBRT, external beam radiation therapy; H&E, hematoxylin and eosin; PCa, prostate cancer; Rad, mice that received 7.5 Gy of radiation; ROS, reactive oxygen species; Sham, mice that received 0 Gy of radiation; TP, lyophilized tomato paste; TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation; TP-Sham, mice that were fed 10% lyophilized tomato powder and received 0 Gy of radiation; TRAMP, TRansgenic Adenocarcinoma of the Mouse Prostate.

### Diet study

The diet study was designed as a 2 × 2 factorial design with diet and radiation treatment as the main variables. Male TRAMP mice were randomly assigned to consume control diet ($n = 35$) or 10% TP ($n = 38$). Power analyses (power = 0.80 and $\alpha = 0.05$) indicated that 15 animals/group would be sufficient to detect a 40% change in oxidative damage in nontumor tissues (24). The primary outcome for this study was changes in apoptosis within the surrounding tissues. Similar to the pilot study, prostates were scanned by ultrasound beginning at 10 wk of age, biweekly (every 2 wk) for longitudinal tumor screening and tumor volume measurement. Mice with detected prostate tumors were weekly scanned ultrasonically to measure tumor volume. Mice were irradiated with 7.5 Gy by a Cobalt-60 source at a dose rate of 0.22 Gy/min (Rad, $n = 19$; TP-Rad, $n = 18$) or 0 Gy (Sham, $n = 16$; TP-Sham, $n = 20$) at 24 wk of age without a detected tumor or once the tumor's volume exceeded 1000 $mm^3$. Mice were euthanized 24 h after radiation or sham treatments based on data from the pilot study.

### Necropsy

TRAMP mice were exsanguinated by cardiac puncture under deep anesthesia followed by cervical dislocation. When possible, the prostate was dissected into individual lobes (anterior, dorsal, lateral, and ventral). Suspected malignant prostate masses (tumors) were dissected from the remaining prostate. Sections of individual prostate lobes, malignant prostate tumors, seminal vesicles, liver, lungs, and epididymal adipose tissue were weighed, snap frozen in liquid $N_2$, and stored at −80°C for future analysis. Sections of the prostate tumors, adjacent prostatic lobes (dorsolateral, ventral, and anterior lobes), visibly enlarged abdominal lymph nodes (e.g., those containing metastases), urethra, bladder, kidney, liver, lung, and small intestine were fixed in 10% neutral buffered formalin for no more than 48 h and held in 70% aqueous ethanol until paraffin embedding. Malignant prostate masses that grew outside of the prostate capsule were considered as tumors within these studies in order to evaluate changes within the tumor. The growing tumor often engulfed portions of adjacent (nonneoplastic) prostate. Accordingly, the amount of tissue available for analysis varied for each animal.

### Histopathology and immunohistochemistry

Tissues selected for evaluation included prostate tumors, adjacent prostatic lobes (dorsolateral, ventral, and anterior lobes), visibly enlarged abdominal lymph nodes (e.g., those containing metastases), urethra, bladder, kidney, liver, lung, and small intestine. Tissues were fixed in 10% neutral buffered formalin for no more than 48 h and stored in 70% ethanol. The small intestine was sliced longitudinally along the antimesenteric border, contents gently washed free and fixed in a "swiss roll" fashion so that the entire tract with all 3 segments (duodenum, jejunum, and ileum) could be examined (25). Tissues were dehydrated and embedded in paraffin. Tissues were sectioned at 4-μm thickness for staining with hematoxylin and eosin (H&E). A board-certified veterinary pathologist (MAW) evaluated the tissues in a blinded fashion to identify morphological changes including cell death (apoptosis and necrosis), edema, and inflammation (or infiltration by scavenger macrophages). The pathologist also evaluated the occurrence and severity of neoplasia in prostate and tumor sections in a blinded fashion as previously described (26). Metastases were confirmed by H&E and SV-40 staining. Cell death scores (apoptosis and necrosis combined) were evaluated within the PCa tumor and selected adjacent tissues (urethra, bladder, small intestine, and liver) in blinded fashion, using a semiquantitative scoring scale (**Supplemental Appendix 1**). Apoptosis was evaluated by cleaved caspase-3 (CC3) immunohistochemical evaluation (Cell Signaling Technology) stained slides. Diffuse pale brown to dark brown cytoplasmic staining was considered positive. Within the prostate and tumor, CC3 was evaluated using a semiquantitative scale (**Supplemental Appendix 2**). The entire small intestine was evaluated for a global estimation of apoptosis at a low magnification in 100-crypt segments as previously validated (27). The number of CC3-positive cells in each segment was counted and averaged for each section of the small intestine.

### Antioxidant analysis

Diet and tissue concentrations of carotenoids and α-tocopherol were extracted and analyzed by HPLC as previously described (28, 29). Approximately 25 mg diet, 300 mg tumor tissue, 200 μL serum, and 100 mg liver tissue were used for analysis. Anterior prostates from 10 mice/treatment condition (1 lobe/mouse) were pooled into 2 individual replicates ($n = 5$/replicate).

### Cytokine analysis

Serum samples were stored at −80°C until the day of analysis. Serum concentrations of IL-1β, IFN-γ, TNF-α, IL-6, IL-10, and IL-17a were measured using a Bio-Plex multiplex assay (Bio-Rad) as previously described (30). Serum concentrations of C-reactive protein (CRP) were assessed by a commercial ELISA kit following the manufacturer's instructions (Abcam). Portions of the liver, epididymal adipose tissue, anterior prostate, and prostate tumor were homogenized in PBS and frozen at −20°C overnight. Tissue samples were thawed and centrifuged at 4°C at 2000 × $g$ for 15 min. TNF-α was quantified in the resultant tissue-derived supernatant by an ELISA following the manufacturer's instructions (Thermo Fisher Scientific).

### Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc.), GraphPad Prism 8.4 for Windows (GraphPad Software), and RStudio Version 1.4.1103 (RStudio). Tissue weights, serum cytokines, and tissue cytokines were compared using 1- or 2-factor ANOVA with multiple-comparison adjustments by Tukey's method. Data were log transformed when assumptions of normality were not met. Histological scores for prostate tumor, nonneoplastic prostate lobes, lungs, liver, kidney, urethra, and regional lymph nodes were assessed using a Kruskal–Wallis test with multiple comparisons by Dunn's test. Histology scores for the small intestine were evaluated using a randomized permutation test for interaction effects using Freedman and Lane's approach with the package Permunco (31). Tissue accumulation of carotenoids was evaluated using a 2-tailed $t$ test. Unless otherwise stated, a $P$ value $< 0.05$ was considered statistically significant. All values are reported as mean ± SEM.

## Results

### Whole-animal and tissue alterations—pilot study

There were no differences between the body weights at euthanasia across the time points that were evaluated (**Supplemental Figure 2**A, B). In addition, there were no differences in the epididymal adipose tissue, lungs, heart, and total prostate weights (**Supplemental Table 2**). After radiation treatment (24 or 72 h after radiation), the mean weight of the spleen decreased by 50% ($P < 0.001$). Mean testicular weight also decreased by 10% after 72 h ($P < 0.01$). Mean PCa tumor weight was numerically 68% lower than sham-treated mice 72 h postirradiation; however, statistical comparisons were not possible (Supplemental Table 2).

By 24 wk of age, 100% of mice developed histologically confirmed prostate adenocarcinoma (**Supplemental Table 3**). Malignant masses that extended out of the prostatic envelope were considered tumors. Histological evaluation of tumors suggested that radiation increased the median cell death scores within the tumor from 1 (0%–1% cell death) in sham-treated mice to 3 (25%–50% cell death) 24 and 72 h postradiation (**Supplemental Table 4**). Statistical evaluation of these scores was not appropriate because of the low number of mice with tumors in the 72-h group ($n = 2$). No significant lesions or morphological changes were noted in the small intestine, bladder, urethra, prostate, or liver with radiation exposure (data not shown). Serum CRP concentrations were 50% lower than in

**TABLE 1** Incidence of adenocarcinoma in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice (diet study)[1]

| | Adenocarcinoma (WD-PD) | | Prostatic lesion score, % total | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | +/total *n* | % | NSL | PIN | WD | MD | PD |
| Diet study | 41/70 | 59 | | | | | |
| Sham | 11/14 | 79 | 0 | 21 | 21 | 7 | 50 |
| TP-Sham | 12/20 | 60 | 0 | 40 | 15 | 0 | 45 |
| Rad | 8/19 | 42 | 0 | 58 | 11 | 11 | 21 |
| TP-Rad | 10/17 | 59 | 0 | 41 | 24 | 0 | 35 |

[1]Values are the number (+) and percentage of mice positive for a designated pathology within each treatment group. Cancer incidence was evaluated by stage by a trained veterinary pathologist. Histologically confirmed adenocarcinoma occurred in 59% of control- and tomato-fed animals (equally distributed by diet). MD, moderately differentiated adenocarcinoma; NSL, no significant lesion; PD, poorly differentiated carcinoma; PIN, prostatic intraepithelial neoplasia; Rad, mice that received 7.5 Gy of radiation; Sham, mice that received 0 Gy of radiation; TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation; TP-Sham, mice that were fed 10% lyophilized tomato powder and received 0 Gy of radiation; WD, well-differentiated adenocarcinoma.

the other treatment conditions 72 h postradiation ($P = 0.005$) (**Supplemental Figure 3A**).

### Whole-animal and tissue alterations—diet study

Unlike the pilot study where 100% of mice developed histologically confirmed prostate adenocarcinoma, 59% of all mice (58% of control-fed and 59% of TP-fed mice) developed PCa by 24 wk (**Table 1**). There were no differences in mean body weight at euthanasia regardless of diet or treatment condition (Supplemental Figure 2C, D). There also were no differences in liver, epididymal adipose tissue, lungs, heart, testes, prostate tumor, individual prostatic lobes, and total prostate weights (**Supplemental Table 5**). For the spleen, there was a main effect of radiation such that irradiated mice had lower mean spleen weights than sham-treated mice ($P < 0.0001$).

### Carotenoid and α-tocopherol content of diet and accumulation in tissues

Supplemental Table 1 describes the ingredients for each diet. **Figure 2** shows the carotenoid composition of the tomato diet, whereas **Table 2** shows tissue carotenoid accumulation. Comparisons for carotenoids were only carried out between TP-Sham and TP-Rad owing to the absence of carotenoids in the control diet. Lycopene was the predominant carotenoid (39 nmol/g, 21 mg/kg, 49% of total carotenoids) in the TP diet. The AIN-93G base for both diets also provided ~120 nmol/g (75 IU) α-tocopherol. The profile of carotenoids in the liver and serum largely reflected the composition of the tomato diet for sham-treated mice. Radiation decreased serum concentrations of lycopene ($P < 0.0001$), phytoene ($P < 0.01$), and α-tocopherol ($P < 0.01$) compared with sham-treated mice by 48%, 26%, and 22%, respectively (Table 2). Although serum lycopene and other antioxidants were lower after radiation, tissue concentrations in the liver and prostate tumor were not modified by radiation. Antioxidants in the prostate were not statistically compared; however, mean α-tocopherol concentrations within the prostate were 42% lower in irradiated mice than in sham-treated mice.

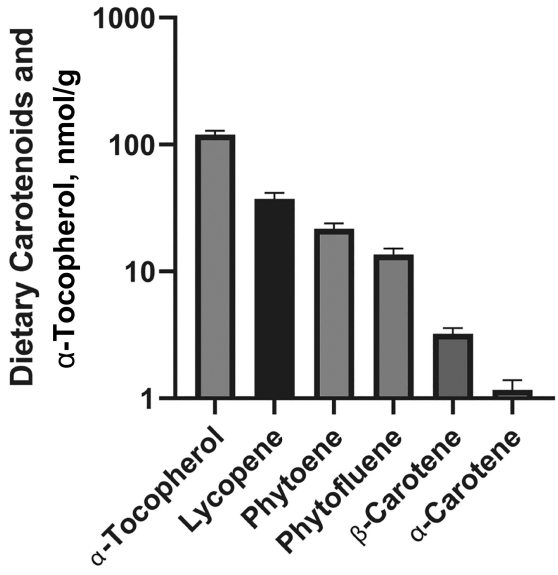


**FIGURE 2** Carotenoid and α-tocopherol content in the tomato powder diet. Data are mean concentration ± SEM, $n = 3$ (means of duplicates in each batch). Data are presented as nmol/g diet. Control diets were not included owing to the lack of carotenoids in these diets.

### Morphologic changes in PCa, prostate, and nonprostatic tissues

There were no significant lesions or morphologic changes, including cell death within the urethra, bladder, small intestine, lungs, or liver, as a result of diet or radiation treatments (data not shown). However, there were substantial foci of necrosis and apoptosis within many prostate tumors that were highly variable in size and distribution across all of the diet and treatment conditions. Often, these foci were in dense clusters with obviously necrotic foci. Within these foci, inflammation contained neutrophils with more subtle infiltration by scavenger macrophages. Both processes were present, with most of the focus being composed of nonidentifiable cell debris, in most foci. Hence, both were combined and scored as "cell death." Cell death scores within the prostate were between 1.5 and 2.5 for all treatment conditions, indicating that, on average, 10%–25% of most tumors had substantial cell death (**Table 3**). Neither diet nor treatment altered the overall proportion of cell death within the tumor ($P = 0.33$).

CC3 expression was used to identify cells in the process of apoptosis. CC3 scores are shown in **Tables 4** (prostate tumors and associated nonneoplastic prostate) and **5** (small intestine). CC3 expression was negligible to absent in all other tissues (urethra, bladder, lung, kidney, and liver) regardless of treatment (data not shown). Within the tumor, the median CC3 scores substantially increased in irradiated mice from 1 in Sham mice to 4 in Rad mice (Table 4) ($P < 0.001$), with >50%

**TABLE 2** Carotenoid and α-tocopherol concentrations in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice fed tomato diets (diet study)[1]

| | *n* | Lycopene[2] | Phytoene | Phytofluene | α-Carotene | α-Tocopherol |
|---|---|---|---|---|---|---|
| Serum | | | | | | |
| TP-Sham | 17 | 865 ± 84*** | 174 ± 12* | 834 ± 94 | n.d. | 67.8 ± 4.7* |
| TP-Rad | 13 | 456 ± 59 | 129 ± 11 | 618 ± 69 | n.d. | 53.1 ± 3.4 |
| Liver | | | | | | |
| TP-Sham | 11 | 16.9 ± 2.4 | 4.64 ± 0.59 | 27.4 ± 3.2 | 0.17 ± 0.06 | 181 ± 21 |
| TP-Rad | 12 | 23.1 ± 2.1 | 5.10 ± 0.74 | 31.4 ± 4.4 | 0.16 ± 0.07 | 188 ± 23 |
| Prostate[3] | | | | | | |
| TP-Sham | 2 | 0.44 ± 0.07 | n.d. | 0.40 ± 0.02 | n.d. | 8.52 ± 0.92 |
| TP-Rad | 2 | 0.72 ± 0.07 | n.d. | 0.46 ± 0.14 | n.d. | 4.96 ± 2.14 |
| Tumor | | | | | | |
| TP-Sham | 4 | 0.28 ± 0.02 | 2.74 ± 0.77 | 0.49 ± 0.08** | n.d. | 18.0 ± 1.9 |
| TP-Rad | 4 | 0.46 ± 0.12 | 3.26 ± 1.32 | 0.90 ± 0.07 | n.d. | 23.0 ± 3.6 |

[1] All values are means ± SEMs, unless otherwise indicated. All concentrations in serum are expressed in nmol/L except for α-tocopherol (μmol/L). All concentrations in liver, prostate, and tumor are expressed in nmol/g. *,**,***Different from TP-Rad (*t* test): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The limit of detection was 0.015 nmol of each carotenoid per gram tissue. Lutein and β-carotene were analyzed, but not detected in any tissue. n.d., not detected (concentration was below the limit of detection); TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation; TP-Sham, mice that were fed 10% lyophilized tomato powder and received 0 Gy of radiation.
[2] Total lycopene (sum of all *trans* and *cis* stereoisomers).
[3] Prostate concentrations are means of 2 pools of 5 anterior prostates (1 lobe/mouse).

of cells in Rad and TP-Rad tumors apoptotic based on CC3 staining. In nonneoplastic prostate, apoptosis was not modified by treatment or diet within the anterior ($P = 0.85$) or ventral prostates ($P = 0.35$). However, the median score for CC3 was altered in the dorsolateral prostate. TP-Sham had lower CC3 scores than Sham and Rad (Table 4) ($P < 0.05$). Likewise, TP-Rad had a trend toward lower CC3 scores than Rad ($P = 0.07$) for nonneoplastic dorsolateral prostate. The lateral lobe of the prostate has been reported to have the highest concentration of carotenoids in the rodent prostate (32).

CC3-positive cells within the small intestine (Table 5) were confined almost exclusively to cryptal enterocytes in all segments. In general, sham-treated mice had very low scores for CC3 expression across all segments of the small intestine regardless of diet. Radiation treatment increased the scores in all 3 segments of the small intestine compared with sham-treated animals ($P < 0.01$). There was a significant effect of diet such that mean scores for CC3-positive cells were lower in TP-fed mice for the cranial duodenum ($P = 0.001$), the caudal duodenum ($P = 0.004$), and the jejunum ($P = 0.04$). There was also a trend for diet to reduce CC3 expression within the ileum ($P = 0.061$). Likewise, there was a significant interaction between diet and radiation treatment within the cranial duodenum ($P = 0.02$) and caudal duodenum ($P = 0.01$). These are the regions of the intestine that absorb carotenoids and other antioxidants (33, 34). Within the duodenums of radiation-treated mice, TP-fed mice had 66.5% fewer CC3-positive cells within the cranial duodenum and 74.7% fewer CC3-positive cells within the caudal duodenum than their radiation-only counterparts. There was no interaction between treatment and diet for the jejunum ($P = 0.23$) or ileum ($P = 0.32$). However, concentrations of carotenoids and antioxidants in these segments are generally expected to be lower than in the duodenum (33, 34).

**TABLE 3** Cell death scores within TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) prostate tumors (diet study)[1]

| | *n* | Mean ± SEM | Median ± SE | *P* value |
|---|---|---|---|---|
| Diet study | | | | 0.33 |
| Sham | 7 | 1.6 ± 0.3 | 2.0 ± 0.5 | |
| TP-Sham | 8 | 1.6 ± 0.3 | 1.5 ± 0.3 | |
| Rad | 4 | 2.3 ± 0.9 | 2.5 ± 1.0 | |
| TP-Rad | 7 | 2.4 ± 0.4 | 2.0 ± 0.5 | |

[1] Values are means ± SEMs and medians ± SEs of the median. There were no significant differences in medians (Kruskal–Wallis test). A score of 0 represents 0%–1% cell death (apoptosis and necrosis); a score of 1 represents ≤10% cell death; a score of 2 represents 10%–25% cell death; and a score of 3 represents 25%–50% cell death. Rad, mice that received 7.5 Gy of radiation; Sham, mice that received 0 Gy of radiation; TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation; TP-Sham, mice that were fed 10% lyophilized tomato powder and received 0 Gy of radiation.

### Inflammatory markers

Four proinflammatory (TNF-α, IFN-γ, IL-17a, and IL-6) cytokines and 1 anti-inflammatory (IL-10) cytokine were measured in the serum. There were no main effects of diet on the measured cytokines. Radiation decreased circulating TNF-α ($P < 0.0001$), IFN-γ ($P < 0.001$), IL-6 ($P < 0.01$), IL-17a ($P < 0.01$), and IL-10 ($P = 0.01$) concentrations by 50%, 35%, 35%, 35%, and 22%, respectively (**Figure 3**A). TNF-α concentrations within the liver ($P = 0.30$), adipose tissue ($P = 0.22$), prostate ($P = 0.27$), and tumor ($P = 0.63$) were not significantly modified by diet or radiation (Figure 3B). Serum CRP concentrations were not modified by diet ($P = 0.24$) (Supplemental Figure 3B).

## Discussion

Males who undergo treatment of PCa often seek information about supplements containing lycopene or tomato components with the goal of improving their therapeutic outcomes. However, to our knowledge no epidemiological or preclinical experiments have evaluated the potential for tomato consumption to modify the PCa and surrounding adjacent tissue response to therapy. The current study addresses a gap in the literature by evaluating the hypothesis that tomato consumption decreases radiation-induced inflammation and apoptosis after radiation within the prostate tumor and other surrounding organs. To our knowledge, no previous studies have evaluated the interactions between tomato consumption

**TABLE 4** CC3 evaluation in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) tissues (diet study)[1]

| | *n* | Mean ± SEM | Median ± SE | *P* value |
|---|---|---|---|---|
| Tumor | | | | 0.0004 |
| Sham | 9 | 1.1 ± 0.4 | 1 ± 0.5[a] | |
| TP-Sham | 7 | 2.8 ± 0.5 | 3 ± 0.5[ab] | |
| Rad | 5 | 4.2 ± 0.5 | 4 ± 0.5[b] | |
| TP-Rad | 7 | 4.0 ± 0.4 | 5 ± 0.4[b] | |
| Dorsolateral prostate | | | | 0.002 |
| Sham | 5 | 1.6 ± 0.2 | 2 ± 0.3[a] | |
| TP-Sham | 10 | 0.8 ± 0.1 | 1 ± 0.2[b] | |
| Rad | 8 | 1.6 ± 0.2 | 2 ± 0.2[a] | |
| TP-Rad | 9 | 1.0 ± 0.0 | 1 ± 0.0[ab†] | |
| Ventral prostate | | | | 0.83 |
| Sham | 4 | 0.5 ± 0.3 | 0.5 ± 0.4 | |
| TP-Sham | 7 | 0.3 ± 0.2 | 0 ± 0.2 | |
| Rad | 6 | 0.3 ± 0.3 | 0 ± 0.4 | |
| TP-Rad | 4 | 0.3 ± 0.3 | 0 ± 0.3 | |
| Anterior prostate | | | | 0.35 |
| Sham | 14 | 1.4 ± 0.4 | 1 ± 0.5 | |
| TP-Sham | 17 | 1.2 ± 0.2 | 1 ± 0.2 | |
| Rad | 14 | 1.2 ± 0.3 | 1 ± 0.3 | |
| TP-Rad | 13 | 0.8 ± 0.2 | 1 ± 0.3 | |

[1]Values are means ± SEMs and medians ± SEs of the median. Medians without a common letter differ (Kruskal–Wallis test followed by Dunn's multiple-comparisons test; $P < 0.05$). †TP-Rad compared with Rad in the dorsolateral prostate trended toward significance ($P = 0.07$). A CC3 score of 0 represents no increase over background (0–1 positive cell/lobular profile); a score of 1 represents a focal increase above background (<25% lobular profiles with 2–5 positive cells/profile); a score of 2 represents "widespread" increases above background (>25% lobular profiles with 2–5 positive cells/profile); a score of 3 represents a "massive" increase above background (>25% lobular profiles with >5 positive cells/profile); a score of 4 represents 50% of lobules affected, with >50% having 6 to ≥10 positive cells, generally associated with foci of cell death and occasional aggregates of positive cells; and a score of 5 represents 50% of lobules affected, with >50% having ≥10 cells/lobule centered around the edges of foci of cell death plus scattered aggregates of positive cells. CC3, cleaved caspase-3; Rad, mice that received 7.5 Gy of radiation; Sham, mice that received 0 Gy of radiation; TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation; TP-Sham, mice that were fed 10% lyophilized tomato powder and received 0 Gy of radiation.

and radiation using a preclinical model. We hypothesized that a diet containing lyophilized TP would reduce apoptosis within carotenoid-containing nonneoplastic tissues without decreasing apoptosis within prostate tumors. Data from this study support our hypothesis that tomato consumption during radiation therapy reduces apoptosis in tissues that contain carotenoids without decreasing apoptosis within the prostate tumor.

About two-thirds of radiation-induced damage is caused indirectly through action of ROS (35). The use of antioxidants during radiation therapy is controversial because some have hypothesized that antioxidants might protect the targeted tissue from the effects of radiation (36–39). Tomato consumption increases carotenoid concentrations in the blood and several tissues (including the prostate), which may result in increased antioxidant protection and decreased inflammation (40, 41). It is possible that lycopene and other tomato carotenoids may decrease the efficacy of EBRT by quenching ROS in the tumor before substantial DNA damage occurs (35, 42). However, TP consumption did not decrease apoptosis and cell death scores in irradiated TRAMP prostate tumors, suggesting that TP did not protect prostate tumors in our study.

We found that some nontumor tissues were less susceptible to the harmful effects of EBRT in mice fed TP. This was most notable in the small intestine within the current study. The composition of tumor interstitial fluid is highly heterogeneous (43). Within the prostate tumor, carotenoid concentrations were low (~50-fold lower than in the liver). This suggests that the prostate tumor is less protected from the effects of radiation partially due to a relatively low concentration of carotenoids that are not evenly distributed through the tumor. Lycopene and other carotenoids have been shown to also reduce angiogenesis within tumors by altering expression of vascular endothelial growth factor (VEGF) and inflammatory markers (44, 45). This would further decrease the ability of the prostate tumor to acquire carotenoids and other antioxidants because these compounds are rapidly quenched during radiolysis.

In the diet study, circulating concentrations of antioxidants (such as lycopene and α-tocopherol) were decreased by 35%–50% 24 h after radiation in TP-Rad compared with TP-Sham. Without an adequate supply of antioxidants from the diet, such as lycopene and α-tocopherol, serum and tissue concentrations would rapidly become depleted, and nonneoplastic tissues could be more heavily damaged through indirect activity of ROS. As injuries in these tissues accumulate, clinical acute and late-stage toxicities may occur leading to digestive, urinary, bowel, or sexual dysfunction (7). Tomato carotenoids are absorbed and accumulate within several tissues (46). TP consumption during radiation (TP-Rad) resulted in lower rates of CC3-positive cells within the cranial (67% lower) and caudal (75% lower) duodenum than for control-fed (Rad) mice ($P < 0.05$). This decrease suggests that TP consumption can reduce radiation-induced damage within the small intestine, which is a common site for acute and late-stage toxicities (7). Although intestinal carotenoids were not directly measured in the current study owing to a lack of tissue, other studies by our group and others have observed carotenoid accumulation within the small intestine (33, 34). Future studies are needed to evaluate the changes in carotenoid content within the small intestine after EBRT.

Dietary preclinical and clinical studies are critical for men who are being treated with EBRT because many men who are diagnosed with PCa choose to improve their diet after diagnosis. Although consumption of fruits and vegetables generally increases after PCa diagnosis, tomato consumption remains relatively constant (<15% increase) after diagnosis (47). Tomatoes are the second most commonly consumed vegetable in the United States, representing 19% of all vegetable consumption (48). To our knowledge, there is currently only 1 small study in humans ($n = 17$) that evaluated the interactions between tomato consumption and EBRT (49). Men were supplemented with 0 mL, 118 mL, 237 mL, or 355 mL tomato juice during radiation therapy (49). The goal of that study was to evaluate the tolerance and adverse events associated with tomato supplementation. No adverse events were noted with tomato consumption, indicating its safety. Although the sample size was small, an important finding was that higher serum lycopene concentrations were associated with less cachexia and improved therapeutic outcomes among patients treated with EBRT (49). Larger clinical and preclinical interventional studies are necessary to determine the role that tomato consumption has during EBRT.

The amount of lycopene within the TP diet of the diet study is achievable through the diet for humans. TP consumption in this study resulted in blood lycopene concentrations that are relevant to humans (12, 50–52). The TP diets in this study contained ~3 mg lycopene/kg body weight. This concentration is necessary in a mouse to achieve blood lycopene

**TABLE 5** CC3 evaluation in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) intestinal segments (diet study)[1]

| | *n* | Mean ± SEM | Diet *P* value | Treatment *P* value | Interaction *P* value |
|---|---|---|---|---|---|
| Cranial duodenum | | | 0.001 | 0.0002 | 0.02 |
| Sham | 5 | 1.3 ± 0.1[a] | | | |
| TP-Sham | 16 | 1.2 ± 0.2[a] | | | |
| Rad | 10 | 43.4 ± 8.3[b] | | | |
| TP-Rad | 8 | 17.6 ± 6.3[a] | | | |
| Caudal duodenum | | | 0.004 | 0.002 | 0.01 |
| Sham | 5 | 1.3 ± 0.1[a] | | | |
| TP-Sham | 16 | 1.1 ± 0.1[a] | | | |
| Rad | 10 | 25.1 ± 5.7[b] | | | |
| TP-Rad | 7 | 6.4 ± 2.4[a] | | | |
| Jejunum | | | 0.042 | 0.002 | 0.24 |
| Sham | 6 | 1.5 ± 0.2[ab] | | | |
| TP-Sham | 16 | 1.1 ± 0.1[a] | | | |
| Rad | 14 | 17.8 ± 3.4[c] | | | |
| TP-Rad | 15 | 10.1 ± 2.8[bc] | | | |
| Ileum | | | 0.061 | 0.006 | 0.31 |
| Sham | 6 | 1.5 ± 0.2[a] | | | |
| TP-Sham | 16 | 1.1 ± 0.1[a] | | | |
| Rad | 14 | 9.4 ± 2.2[b] | | | |
| TP-Rad | 15 | 4.8 ± 1.3[b] | | | |

[1] Values are means ± SEMs unless otherwise indicated. Means without a common letter differ ($P < 0.05$). Values for the mean represent the number of cells expressing CC3 per 100 crypts. Data were evaluated by Freedman–Lane's permutation test (5000 permutations). CC3, cleaved caspase-3; Rad, mice that received 7.5 Gy of radiation; Sham, mice that received 0 Gy of radiation; TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation; TP-Sham, mice that were fed 10% lyophilized tomato powder and received 0 Gy of radiation.

concentrations similar to those of humans owing to the poor absorption of carotenoids in rodents (53). The ranges of blood lycopene concentrations found in this and similar studies correlate well with blood concentrations in American men over the ranges that are associated with a significant reduction in risk of lethal PCa in the Health Professionals Follow-Up Study (HPFS) prospective cohort trial and other studies (45, 54, 55). A human equivalent dose of 3 mg/kg in mice translates to 17 mg/d (0.24 mg/kg) (56). This could be achieved with a half-serving of tomato sauce (1/4 cup, 60 g) per day. Lycopene and other carotenoid absorption increases substantially with cooking (thermal processing) or lipid content within the diet (57, 58). Men accumulate carotenoids in the prostate, and prostate lycopene concentrations are positively correlated with blood concentrations (59).

Among the possible animal models available, we selected the TRAMP model. This model is well established and exhibits a predictable histological progression from low-grade hyperplasia to poorly differentiated adenocarcinoma and local as well as distant metastasis, resembling development of the human PCa (60). Neuroendocrine tumors arise in a high proportion of poorly differentiated TRAMP tumors and a smaller proportion of human PCa. These tumors are aggressive and are most frequently treated with radiation, making the TRAMP model particularly relevant for neuroendocrine PCa (61). We, and our colleagues, have shown that TRAMP mouse tissues (including the prostate tumor) have similar carotenoid biodistribution patterns to humans (29, 52, 62). Accordingly, carotenoids and other dietary tomato compounds are able to be absorbed and accumulate within tissues in a similar manner to how they are in humans. These factors suggest that the TRAMP model is an excellent choice to evaluate the capacity for carotenoids to alter radiation-induced damage.

These studies were informed by the outcomes of a pilot study. Within the pilot study, dosimeters confirmed the dose of radiation received, and radiation-sensitive tissues (e.g., spleen and testes) rapidly decreased in weight postradiation. Serum CRP concentrations and tumor weights appeared to also decrease after 72 h. Cell death scores remained similar at 24 and 72 h postradiation. CC3 signaling cascades begin within hours after radiation exposure (27), and secondary necrotic/phagocytic programs are initiated soon after. By 72 h, we hypothesized that most acute apoptosis was beginning to resolve. Therefore, endpoints were evaluated 24 h postradiation to evaluate the acute effects of TP consumption. In the diet study, TP provided a bioavailable vehicle for multiple bioactive compounds; TP is thermally processed and contains dietary lipids that can enhance the absorption of carotenoids. TP is readily available, convenient, and inexpensive for consumers. Preliminary data in humans also indicate that TP supplementation during EBRT is well tolerated with no notable side effects (49).

These studies also have limitations that should be considered. A single radiation dose of 7.5 Gy was used. Many acute toxicities occur weeks after the onset of the treatment (63, 64). It is possible that the accumulation of damage from several doses might enhance damage to the tumor and surrounding tissue compared with a single dose. As the dose of radiation increases, oxidative damage within the tumor and surrounding tissue increases and the number of required fractions (doses) decreases (65–68). Future studies will be needed to determine if TP alters tumor cell death over a prolonged period of time. Still, it is notable that there was a decrease in deleterious effects of radiation on normal tissue without protecting tumor tissue, which is difficult to accomplish with dietary radioprotectants. We hypothesize that this would remain consistent through the course of radiation therapy. Fewer mice developed tumors

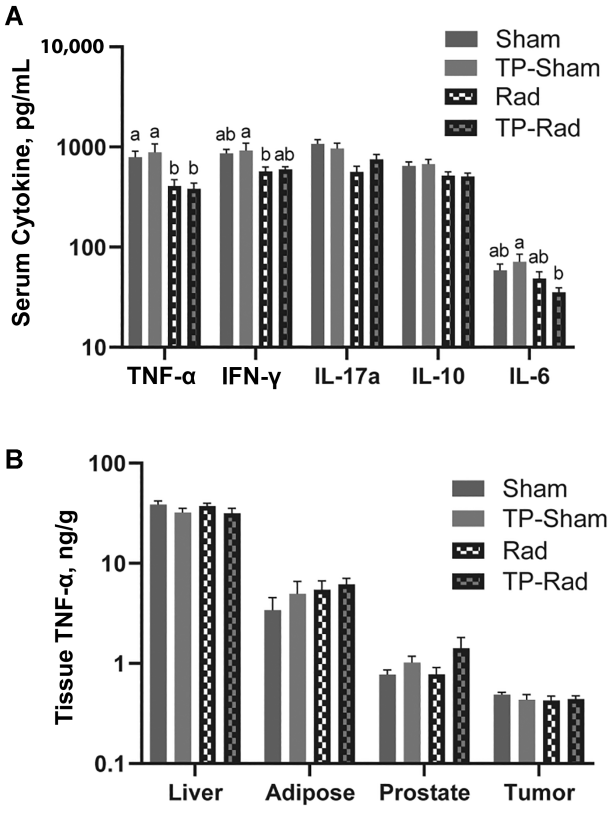


**FIGURE 3** Serum and tissue cytokine concentrations in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice. (A) Serum concentrations of cytokines ($n$ = 8–13/group). (B) Tissue concentrations of TNF-α ($n$ = 6/group). Data are mean ± SEM. Means without a common letter differ (1-factor ANOVA followed by Tukey's post hoc test, $P < 0.05$). Rad, mice that received 7.5 Gy of radiation; Sham, mice that received 0 Gy of radiation; TP-Rad, mice that were fed 10% lyophilized tomato powder and received 7.5 Gy of radiation.

within the diet study than in the pilot study. Although we have observed similar rates of cancer incidence in previous studies (51, 52), the median values for cell death scores were very similar. We do not believe the lower incidence in the diet study influenced any of the results. In addition, this study only tested a single concentration of dietary TP throughout the life span. These results are more generalizable to a man who consumes TP through his life span rather than one that changes his dietary pattern after PCa diagnosis. Although multiple doses were not evaluated, men accumulate lycopene in the prostate with moderate doses and short periods of tomato consumption (62). As previously mentioned, 1 small study found that higher serum lycopene concentrations were associated with less cachexia and improved therapeutic outcomes among patients treated with EBRT (49). Future studies should evaluate the effectiveness of consuming TP at the time of diagnosis (tumor detection) to determine if TP is effective as a dietary therapeutic intervention.

To our knowledge, no other preclinical studies have evaluated the potential for tomato consumption to alter the response to EBRT within the tumor and within the surrounding tissues. This work provides preliminary data that tomato consumption with radiation therapy does not diminish the damage occurring within prostate tumors. Importantly, these data suggest that TP modifies the detrimental effects of radiation in other tissues (especially the small intestine). Reduced damage in nonneoplastic tissues reduces the number of adverse effects that may be experienced by the patient. Future preclinical studies should evaluate the potential for dietary tomatoes and lycopene to alter apoptosis with multiple doses of radiation. Future clinical studies should also evaluate the concentration of lycopene in the blood to determine whether increased circulating lycopene correlates positively or negatively with treatment-related outcomes.


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