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Dietary Tomato or Lycopene Do Not Reduce Castration-Resistant Prostate Cancer Progression in a Murine Model

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ABSTRACT

Background: Dietary tomato products or lycopene protect against prostate carcinogenesis, but their impact on the emergence of castration-resistant prostate cancer (CRPC) is unknown.

Objective: We hypothesized that tomato or lycopene products would reduce the emergence of CRPC.

Methods: Transgenic adenocarcinoma of the mouse prostate (TRAMP) mice were castrated at 12–13 wk and the emergence of CRPC was monitored by ultrasound in each study. In Study 1, TRAMP mice ($n = 80$) were weaned onto an AIN-93G-based control diet (Con-L, $n = 28$), a 10% tomato powder diet (TP-L, 10% lyophilized w/w, $n = 26$), or a control diet followed by a tomato powder diet after castration (TP-Int1, $n = 26$). In Study 2, TRAMP mice ($n = 85$) were randomized onto a control diet with placebo beadlets (Con-Int, $n = 29$), a tomato diet with placebo beadlets (TP-Int2, $n = 29$), or a control diet with lycopene beadlets (Lyc-Int, $n = 27$) following castration (aged 12 wk). Tumor incidence and growth were monitored by ultrasound beginning at an age of 10 wk. Mice were euthanized 4 wk after tumor detection or aged 30 wk if no tumor was detected. Tissue weights were compared by ANOVA followed by Dunnett's test. Tumor volumes were compared using generalized linear mixed model regression.

Results: Ultrasound estimates for the *in vivo* tumor volume were strongly correlated with tumor weight at necropsy ($R^2 = 0.75$ and 0.94, $P < 0.001$ for both Studies 1 and 2, respectively). Dietary treatments after castration did not significantly impact cancer incidence, time to tumor detection, or final tumor weight.

Conclusions: In contrast to studies of *de novo* carcinogenesis in multiple preclinical models, tomato components had no significant impact on the emergence of CRPC in the TRAMP model. It is possible that specific mutant subclones of prostate cancer may continue to show some antiproliferative response to tomato components, but further studies are needed to confirm this. *J Nutr* 2020;00:1–10.

Keywords: castration-resistant prostate cancer, tomato, lycopene, androgen deprivation therapy, TRAMP

Introduction

Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in the USA (1). Androgen deprivation therapy (ADT) has been the primary therapy for advanced and metastatic PCa for over 70 y (2). Historically, ADT was performed by surgical castration following the discovery that testosterone was critical to prostate growth and function in laboratory models (3, 4). In recent decades, ADT has been increasingly accomplished by pharmacologic agents and integrated into effective multimodality treatment plans for locally advanced and high-grade localized prostate cancer, and in salvage regimens for local recurrence following prostatectomy (2, 5). This has led to an improvement in quality of life, sexual function, and life expectancy (2, 5). Unfortunately, ADT alone is rarely curative as genetic instability within the cancer cells leads to the emergence of mutant subclones that progress in spite of castrate serum concentrations of testosterone (6). This late and often lethal phenotype is termed castration-resistant prostate cancer (CRPC) (6).

Following 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

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as having a protective effect on prostate cancer activity. The consumption of tomatoes or their predominant carotenoid, lycopene, has been associated with lower PCa risk in many epidemiological studies (7, 8). Interestingly, increased tomato or lycopene consumption in epidemiological cohorts appears to have a greater impact on lethal or aggressive PCa (9–11). In agreement with the human epidemiological evidence, studies in multiple rodent models support the hypothesis that dietary tomato or lycopene reduce *de novo* prostate carcinogenesis (12–14). However, the potential efficacy of dietary tomato or lycopene as a component of an integrated treatment plan to reduce the progression of CRPC has not been thoroughly investigated in experimental systems.

Based on the epidemiological and preclinical evidence for PCa incidence, many men with PCa undergoing ADT or with CRPC might choose to consume lycopene supplements without evidence from definitive phase III human trials. Although some groups have explored the activity of tomato carotenoids on the growth of androgen-insensitive PCa xenografts (15–17), these short-term studies in models of tumorigenesis do not recapitulate the complex and multiple pathways involved in the malignant transition from androgen-sensitive to the castration-resistant state. Additionally, the use of pharmacological doses of lycopene, far beyond what is relevant to the diet, is a concern because little is known regarding the risks of such intake in humans (18, 19). Although these data suggest that dietary tomato or lycopene may provide a benefit to men with advanced androgen-sensitive PCa, a lack of preclinical data on which to base more definitive trials remains a gap in the literature.

We sought to address this hypothesis by investigating whether lifelong tomato consumption, a later dietary tomato intervention, or a later dietary lycopene intervention would be effective in reducing the emergence and growth of CRPC tumors in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. To investigate this hypothesis, we conducted 2 studies. Study 1 investigated the impact of lifelong or postcastration tomato interventions on CRPC incidence and progression in the TRAMP model. Study 2 evaluated the impact of postcastration tomato or lycopene interventions on TRAMP CRPC incidence and progression. To our knowledge, this is the first report to evaluate the efficacy of dietary tomato or lycopene combined with castration (as a model of ADT) to reduce the incidence and progression of CRPC in a rodent model.

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Abbreviations used: ADT, androgen deprivation therapy; Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; CRPC, castration-resistant prostate cancer; H&E, hematoxylin and eosin; Lyc, lycopene; Lyc-Int, control diet with lycopene beadlets provided after castration; PCa, prostate cancer; TP, lyophilized tomato paste; TP-Int, TP provided following castration; TP-L, lifelong consumption of TP; TRAMP, transgenic adenocarcinoma of the mouse prostate.

TABLE 1 Composition of experimental diets

	g/kg diet		
	Control	10% Tomato	Lycopene
Corn starch	390	363	390
Maltodextrin	130	105	130
Sucrose	98	97	98
Casein	196	177	196
Cellulose	49	41	49
AIN-93 G mineral mix	34	34	34
AIN-93 G vitamin mix	10	10	10
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
Soybean oil	70	68	70
Lyophilized tomato paste	0	100	0
10% Lycopene beadlets	0	0	0.47
Placebo beadlets[1]	0.47	0.47	0
Water	18	0	18
kcal/g diet[2]	3.9	3.8	3.9

[1] Placebo beadlets were included only in the control and tomato diets of Study 2.
[2] Calculated.

Methods

Diets

Tomato paste (Contadina®) was purchased from a local supermarket in September 2014 and July 2015 for Study 1 and in April 2016 (Study 2); followed by lyophilization in a VirTis Freezemobile 12SL/Unitop 600 SL freeze dryer (SP Scientific). The dried yield was ~25% of wet mass. Lyophilized tomato paste (TP) was ground to a fine powder in a tabletop food processor, transferred to resealable gallon bags (air removed), and kept in the dark at −20°C until diet mixing.

Two experimental diets were used in Study 1: a powdered, AIN-93G-based control diet and the same diet modified to contain 10% (w/w) TP. In Study 2, similar control and tomato diets were used with the addition of placebo beadlets (0.47 g/kg diet; DSM). Study 2 also included a powdered AIN-93G-based control diet containing lycopene beadlets (Lyc) (0.47 g of 10% lycopene beadlets/kg diet; DSM). The composition of each diet is described in **Table 1**. Ingredients were mixed using a commercial mixer (Hobart). Proximate analysis was performed on the 100% tomato paste powder and diet formulas were balanced for total energy, carbohydrates, protein, fat, fiber, and moisture. New diets were formulated every 1.5–2 mo. Seven (Study 1) or 6 (Study 2) batches of the 10% tomato diet were made throughout the course of the study and each was analyzed for carotenoid content by HPLC.

Mouse breeding, genotyping, and housing

The University of Illinois Laboratory Institutional Animal Care and Use Committee reviewed and approved all experimental procedures (Study 1, protocol number 14,296; Study 2, protocol number 16,078). Male C57BL/6-Tg(TRAMP)8247Ng/J (C57BL/6 TRAMP$^{+/-}$), female C57BL/6 J, and female FVB/NJ mice were purchased from The Jackson Laboratory. A breeding colony was maintained with crosses of C57BL/6 J females and C57BL/6 TRAMP$^{+/-}$ males. Male F_1 offspring of FVB/NJ females and C57BL/6 TRAMP$^{+/-}$ males were used for the study. Tail DNA of pups was isolated with Extract-N-Amp™ Tissue PCR kits (Sigma-Aldrich) and mice were genotyped to confirm transgene presence. Males carrying the probasin: SV40-Tag transgene (hereafter referred to as TRAMP mice) were weaned aged 3 wk and enrolled into the study via rolling admission. Mice were housed under controlled conditions (12-h light/dark cycle, 22åC, 55% humidity), weighed weekly, and diet was added 3 times per week.

Study 1. Timing of tomato feeding

TRAMP mice were acclimated to the AIN-93 G control diet from weaning aged 3 to 4 wk and randomized to consume the control diet

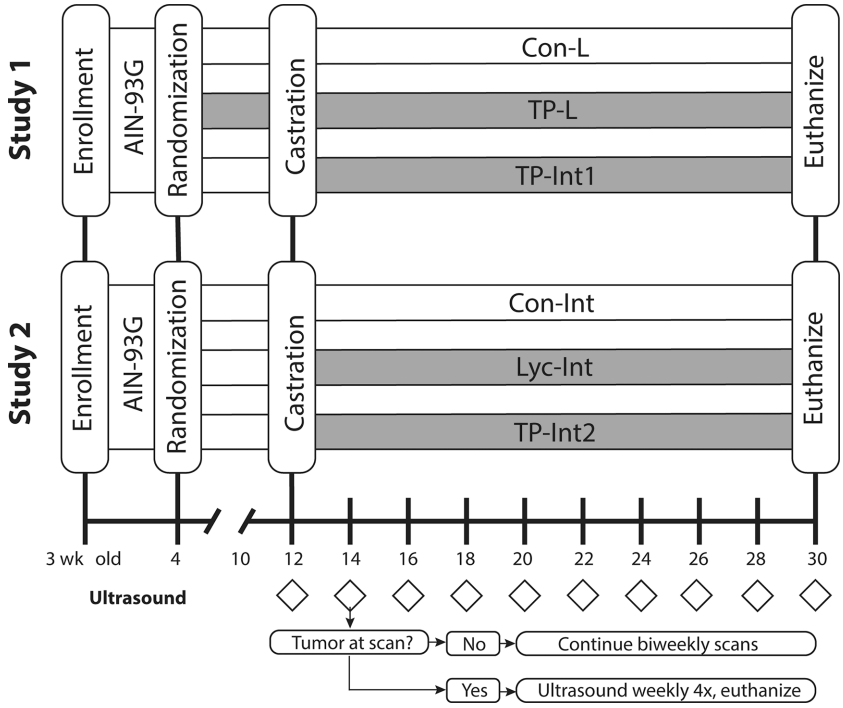

FIGURE 1 Study designs. TRAMP mice were randomized onto dietary treatment groups after weaning (4 wk) or after castration. Study 1 focused on the timing of tomato consumption, whereas Study 2 focused on the impact of the bioactive as an intervention to reduce emergence or growth of CRPC tumors. In both studies, prostates were monitored biweekly for tumor occurrence by ultrasound beginning aged 10 wk. After tumor detection, mice were scanned 4 additional times (+4 weekly ultrasound scans) to track changes in tumor volume. Mice without tumors detected by ultrasound were euthanized aged 30 wk. Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; CRPC, castration-resistant prostate cancer; Lyc-Int, control diet with lycopene beadlets provided after castration; TP-Int1, control diet followed by a tomato powder diet after castration; TP-Int2, a tomato diet with placebo beadlets; TP-L, lifelong consumption of TP; TRAMP, transgenic adenocarcinoma of the mouse prostate.

(Con, $n = 54$) or 10% TP (TP-L, $n = 26$). Following castration aged between 12 and 14 wk, mice were switched from the control diet to an intervention of 10% TP (TP-Int1, $n = 26$) or remained on the control diet (Con-L, $n = 28$).

Study 2. Bioactive comparison

TRAMP mice were acclimated to the AIN-93 G control diet from weaning aged 3 wk until castration aged 12 wk. Following castration, TRAMP mice consumed dietary treatments of control diet with placebo beadlets (Con-Int, $n = 29$), an AIN-93 G diet modified to contain 10% TP with placebo beadlets (TP-Int2, $n = 29$), or the control diet with lycopene beadlets (Lyc-Int, $n = 27$).

Castration surgery

Mice were surgically castrated between 12 and 14 wk under inhalation isoflurane for general anesthesia on a heated platform. The mean age at castration was 13.13 ± 0.06 wk in Study 1 and 12.10 ± 0.03 wk in Study 2. At this age, TRAMP mice exhibit nearly 100% incidence of high-grade prostatic intraepithelial neoplasia or microscopic well- to moderately differentiated adenocarcinoma (20, 21). Subcutaneous injections of an analgesic (buprenorphine, 0.05 mg/kg or carprofen, 5 mg/kg) were given pre- and postsurgery. Figure 1 displays the study designs with diet interventions, castration, and necropsy for both studies.

In vivo ultrasound tumor screening and measurement

Beginning aged 10 wk, biweekly (every 2 wk) *in vivo* ultrasound imaging was used for longitudinal screening and tumor volume measurement. Inhalation isoflurane was used for general anesthesia. Ultrasonic scans were obtained through the ventral body wall while in dorsal recumbency on a heated table using the Vevo 2100 preclinical ultrasonic imaging platform (VisualSonics, Inc.). Scans were conducted in three-dimensional (3D) B-mode, and frames were collected in a caudal to cranial direction at intervals of approximately 0.152 mm. Serial 2D image slices were used to generate prostatic or tumor volume estimates as previously described (22). Mice with prostate tumors identified aged 14 wk or later were switched from biweekly ultrasound screening to weekly ultrasound scans in order to measure CRPC tumor volume.

Necropsy

Mice were euthanized for necropsy based upon the following criteria: *1*) a moribund clinical status, *2*) a 4-wk time period after a tumor mass was detected by ultrasound, *3*) a tumor volume exceeding 5000 mm^3, or *4*) aged 30 wk with no tumor detected by ultrasound. Study 1 mice were euthanized by CO_2 asphyxiation under isoflurane-induced general anesthesia, followed by cervical dislocation. Study 2 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. Individual prostate lobes, malignant prostate tumors, seminal vesicles, liver, lungs, and epididymal adipose tissue were weighed and snap frozen in liquid N_2 and stored at −80°C for future analysis. Gross metastases to the lungs, liver, kidneys, urethra, and regional lymph nodes (medial iliac and lumbar aortic, when present) were identified by visual inspection, and tissues were fixed in 10% neutral-buffered formalin for 12 to 24 h and held in 70% aqueous ethanol until paraffin embedding.

Histopathology and immunohistochemistry

Tissues were embedded in paraffin and 4-μm thick sections were stained with hematoxylin and eosin (H&E). A blinded examiner (SKC or MAW) evaluated the extent and severity of neoplasia in prostate and tumor sections as previously described (23). Metastases were confirmed by H&E and SV-40 staining, and the emergence of poorly

differentiated cancer exhibiting a stereotypic neuroendocrine phenotype was determined by staining against synaptophysin (ABCAM).

Carotenoid measurement

Diet and tissue carotenoids were extracted and analyzed by HPLC as previously described (24, 25). Approximately 25 mg diet, 300 mg tumor tissue, 200 μL serum, and 100 mg liver tissue were used for analysis. Carotenoids in the serum were analyzed by HPLC-tandem MS as previously described (26) in Study 1 and by HPLC in Study 2 (24, 25). Due to castration, anterior prostates atrophied and were too small for individual assay. Thus, anterior prostates from 8 to 12 mice (1 lobe per mouse) were pooled to achieve a quantifiable signal. Anterior prostates from 8 and 12 mice per dietary treatment(1 lobe per mouse) in Study 1 were pooled into 2 individual replicates per treatment. Anterior prostates from 2 and 5 mice per dietary treatment (1 lobe per mouse) were pooled in Study 2.

Statistical analysis

Parallel statistical analyses were conducted for both studies. SAS (version 9.4; SAS Institute) was used for statistical analyses. In total, 80 mice from Study 1 (Con-L, $n = 26$; TP-L, $n = 28$; TP-Int1, $n = 26$) and 85 mice from Study 2 (Con-Int, $n = 29$; TP-Int2, $n = 29$; Lyc-Int, $n = 27$) were included in the final analysis. Descriptive statistics of mouse characteristics such as enrollment age, age at castration, age at euthanasia, weight at euthanasia, occurrence and sites of lesions were obtained using means and SE for quantitative variables and frequencies and percentages for dichotomous variables. Carotenoid accumulation was compared between carotenoid-containing treatments by t test. Body weight at necropsy and organ weights were assessed by ANOVA with multiple comparisons correction by Dunnett's test. Cancer incidence was assessed by Fisher's exact test between the control group and each treatment group. Survival curves were generated using product-limit estimation, with time from castration to appearance of ultrasound-detected tumor treated as the duration of tumor-free survival. We tested for significance of the differences in the survival rates between treatments by employing the log-rank test with PROC LIFETEST. To control for age and weight as covariates, proportional hazards regression using PROC PHREG was performed.

In both studies, 56 mice developed lesions that were detectable by ultrasound with weekly tumor volume measurements. Due to the rapid rate of weekly volume increase and heterogeneity of data, tumor volumes were transformed by natural logarithms. Generalized linear mixed model regression using PROC GLIMMIX was employed to examine differences between the treatment groups for rate of tumor growth during the 5 consecutive weeks of tumor volume monitoring. The model was fitted to the data assuming a lognormal distribution of the outcome. A random intercept and slope, treatment effects as well as the interaction of treatment and time were included in the model. The latter term represented the differences in the rate of tumor growth (slopes) between treatment groups. Experimental units (mice) were nested within treatment group. Age and weight at castration were included in the model as covariates. Pairwise comparisons across treatment groups were conducted using the LSMESTIMATE statement and the P values were adjusted for multiple comparisons using the Sidak test (27). We performed sensitivity analysis in the regression using generalized linear mixed models in 2 ways: *1*) by considering a weighted least squares estimation of the parameter models and *2*) by removing influential observations based on studentized residuals. Differences in the tumor weight at euthanasia, week 0 tumor volume, week 4 tumor volume, and the final nonmissing tumor volume among the treatment groups were evaluated through generalized linear regression, which was conducted using PROC GLMSELECT where age and weight at castration were considered as potential confounders. Sensitivity analyses were conducted for both studies by using a weighted least squares approach and by removing extreme observations. Finally, metastases were not statistically assessed due to insufficient power to evaluate these endpoints. The primary outcomes for this study were cancer incidence, time to tumor detection, tumor growth rate, final tumor volume, and tumor weight. Unless otherwise stated, $P < 0.05$ was considered statistically significant. All values are reported as mean ± SEM.

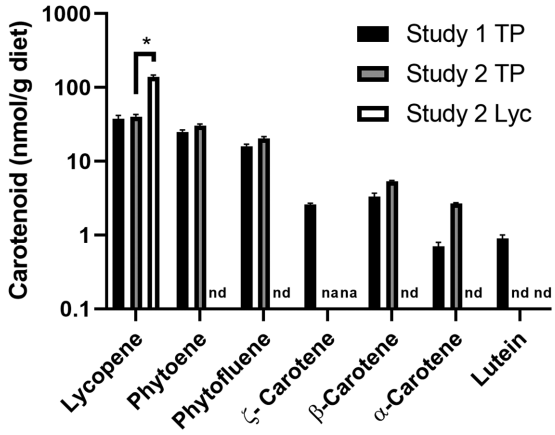

FIGURE 2 Carotenoid composition of the tomato and lycopene diets. Data are mean concentration ± SEM across 6–7 diet batches (means of 2–3 replicates/batch). Data are presented as nmol/g diet. Four carotenoids (ζ-carotene, β-carotene, α-carotene, and lutein) each constituted <10% of total carotenoids. n.d., not detected, carotenoid concentration was below the limit of detection (0.005 nmol carotenoid/g diet); n.a., not analyzed. * means differ, $P < 0.001$ (t test). Lyc, lycopene; TP, lyophilized tomato paste.

Results

Carotenoid content of diet and accumulation in tissues

The carotenoid composition of the tomato and lycopene diets are shown in **Figure 2**. Lycopene was the predominant carotenoid (~40 nmol/g, ~20 mg/kg, ~50% of total carotenoids) in the TP diets for both studies. Within the Lyc-Int diet, the lycopene content was 139 nmol/g (75 mg/kg). No significant differences in tissue carotenoid accumulation were observed between tomato treatments in Study 1 (**Table 2**). Similarly, no significant differences in tissue lycopene accumulation were observed between Lyc-Int and TP-Int2 interventions in Study 2. Although the liver and serum carotenoid profile largely reflected the dietary carotenoid composition, we did not detect phytoene in prostate or tumor tissue, as our laboratory has previously reported for TRAMP mice (20).

Animal characteristics

TRAMP mice were enrolled onto each study aged 4.0 ± 0.1 and 4.2 ± 0.1 wk in Studies 1 and 2, respectively. Mice in Study 1 were a week older at castration than Study 2 (13.1 ± 0.1 wk compared with 12.1 ± 0.1 wk). There were no differences between the body weights at euthanasia across treatment groups in either study (**Supplemental Figure 1**). Additionally, there were no differences in the weight of the prostate tumor, liver, epididymal adipose tissue, lungs, individual prostatic lobes, and total prostate weight within studies (**Supplemental Table 1**).

Tumor incidence and metastases

Cancer incidence is displayed in **Table 3**, which shows 76% of the animals in Study 1 and 77% of the animals in Study 2 developed histologically confirmed moderately or poorly differentiated adenocarcinoma with no significant differences

TABLE 2 Carotenoid accumulation in tomato- and lycopene-fed castrated TRAMP mice

		n	Lycopene[1]	Phytoene	Phytofluene	ζ-Carotene	α-Carotene
	Study 1						
	TP-L	6	569 ± 98[2]	69.1 ± 10.7	142 ± 44	n.a.[3]	n.a.
Serum	TP-Int1	6	551 ± 97	59.1 ± 12.3	136 ± 9	n.a.	n.a.
nmol/L	Study 2						
	TP-Int2	23	285 ± 22	110 ± 7	242 ± 27	n.a.	n.d.
	Lyc-Int	21	346 ± 35	n.d.[4]	n.d.	n.a.	n.d.
	Study 1						
	TP-L	12	15.7 ± 2.1	5.6 ± 0.9	10.5 ± 1.5	1.7 ± 0.2	0.03 ± <0.01
Liver	TP-Int1	11	17.4 ± 2.1	5.7 ± 0.7	11.2 ± 1.5	1.6 ± 0.2	0.03 ± <0.01
nmol/g	Study 2						
	TP-Int2	29	10.1 ± 0.5	4.7 ± 0.5	13.8 ± 1.1	n.a.	n.d.
	Lyc-Int	25	8.5 ± 1.1	n.d.	n.d.	n.a.	n.d.
	Study 1						
	TP-L	2[5]	0.38 ± 0.09	n.d.	0.15 ± 0.02	0.34 ± 0.07	n.d.
Prostate	TP-Int1	2	0.37 ± 0.04	n.d.	0.12 ± 0.01	0.32 ± 0.03	n.d.
nmol/g	Study 2						
	TP-Int2	1[5]	1.50	n.d.	0.51	n.a.	n.d.
	Lyc-Int	1	0.68	n.d.	n.d.	n.a.	n.d.
	Study 1						
	TP-L	3	0.09 ± 0.01	n.d.	0.04 ± 0.01	0.04 ± <0.01	n.d.
Tumor	TP-Int1	4	0.12 ± 0.02	n.d.	0.13 ± 0.04	0.06 ± 0.01	n.d.
nmol/g	Study 2						
	TP-Int2	6	0.07 ± 0.03	n.d.	0.14 ± 0.02	n.a.	n.d.
	Lyc-Int	6	0.10 ± 0.03	n.d.	n.d.	n.a.	n.d.

[1] Total lycopene (sum of all *trans* and *cis* stereoisomers).
[2] All values represent the mean ± SEM. By t test, there were no statistically significant differences between TP treatments (Study 1) in tissue accumulation of any carotenoid. In Study 2, no significant differences were observed between lycopene concentrations TP-Int2 and Lyp-Int.
[3] n.a., not analyzed.
[4] n.d., not detected. Concentration was below the limit of detection. 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.
[5] Prostate concentrations from Study 1 (TP-L and TP-Int1) are means of 2 pools of 8–12 mice each, whereas prostates for Study 2 (TP-Int2 and Lyc-Int) are the mean of 2–5 animals that were pooled.
Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; CRPC, castration-resistant prostate cancer; Lyc, lycopene; Lyc-Int, control diet with lycopene beadlets provided after castration; TP, lyophilized tomato paste; TP-Int1, control diet followed by a tomato powder diet after castration; TP-Int2, a tomato diet with placebo beadlets; TP-L, lifelong consumption of TP; TRAMP, transgenic adenocarcinoma of the mouse prostate.

between treatment conditions ($P = 0.82$ in Study 1 and $P = 0.56$ in Study 2). Expression of neuroendocrine features represented by synaptophysin immunohistochemistry, was expressed in 33% or 42% in the tumors from Studies 1 and 2, respectively, with no significant differences by treatment group. Metastatic spread was visually assessed at necropsy and lesions were confirmed by pathology. For both studies, the statistical analysis of distant metastatic disease was not possible for any site due to the low incidence of metastases observed (**Table 4**). The most common site of metastases was to the lymph node, which occurred in 4–30% of all animals. Approximately 78% of the

TABLE 3 Incidence of histologically confirmed prostate adenocarcinoma or neuroendocrine carcinoma

	Adenocarcinoma (WD-PD)[1]		Prostatic lesion score (% total)[2]					Neuroendocrine carcinoma[1]	
Treatment	+/total *n*	%	NSL	PIN	WD	MD	PD	+/total *n*	%
Study 1									
Con-L	19/25	76	16	8	8	4	64	4/16	25
TP-Int1	18/23	78	17	4	4	13	61	5/13	38
TP-L	17/22	77	18	5	5	27	45	4/10	40
Study 2									
Con-Int	21/27	78	22	0	0	4	74	9/21	29
TP-Int2	22/28	79	18	4	0	0	79	14/22	55
Lyc-Int	20/27	74	26	0	0	11	63	8/19	44

[1] Fisher's exact test between control and respective treatment were not significant for adenocarcinoma and neuroendocrine incidence in Study 1 (Con-L, TP-Int1, TP-L) or Study 2 (Con-Int, TP-Int2, Lyc-Int).
[2] Cancer incidence was evaluated by stage by a trained veterinary pathologist. NSL, no significant lesion; PIN, prostatic intraepithelial neoplasia; WD, well-differentiated adenocarcinoma; MD, moderately differentiated adenocarcinoma; PD, poorly differentiated carcinoma.
Data are provided as the number (+ and %) of mice positive for a designated pathology within each treatment group.
Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; Lyc-Int, control diet with lycopene beadlets provided after castration; TP, lyophilized tomato paste; TP-Int1, control diet followed by a tomato powder diet after castration; TP-Int2, a tomato diet with placebo beadlets; TP-L, lifelong consumption of TP.

TABLE 4 Incidence of histologically confirmed distant metastases

		Lymph nodes		Liver		Lungs		Kidney	
Treatment	*n*[1]	+[2]	%	+	%	+	%	+	%
Study 1									
Con-L	25	5	20	2	8	7	28	5	20
TP-Int1	23	1	4	0	0	1	5	1	5
TP-L	23	7	30	0	0	1	4	1	4
Study 2									
Con-Int	29	5	17	1	3	2	7	1	3
TP-Int2	29	8	28	1	3	2	7	2	7
Lyc-Int	28	7	25	1	4	3	11	4	14

[1] *n*, total mice available for comparison within each treatment group.
[2] Data are provided as the number (+ and %) of mice positive for a designated pathology within each treatment group.
Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; Lyc-Int, control diet with lycopene beadlets provided after castration; TP, lyophilized tomato paste; TP-Int1, control diet followed by a tomato powder diet after castration; TP-Int2, a tomato diet with placebo beadlets; TP-L, lifelong consumption of TP.

metastases in Study 1 and 95% of the metastases in Study 2 stained positive for SV-40.

In vivo CRPC tumor growth

In Studies 1 and 2, 70% and 66% of mice, respectively, developed lesions that were detected by ultrasound and were eligible for *in vivo* growth analyses. Overall, ultrasound estimates for *in vivo* tumor volume were strongly correlated with tumor weight at necropsy ($R^2 = 0.76$ and 0.94, $P < 0.001$ for both Studies 1 and 2, respectively) (**Figure 3**A and B). For both Study 1 and Study 2, no significant differences in tumor-free survival between the treatment groups (time to appearance of the first ultrasound-detected lesion) from the time of castration (aged 12–14 wk) were noted in Study 1 (log rank $P = 0.91$) and Study 2 (log rank $P = 0.70$). These results remained the same even after using proportional hazards regression controlling for age and weight at castration.

The initial tumor volume (week 0) was not significantly different between treatment groups for mice on either study ($P = 0.22$ and $P = 0.28$ for Studies 1 and 2, respectively). The mean tumor volume at detection was ~50 mm^3 in Study 1 (Figure 3C) and 20 mm^3 in Study 2 (Figure 3D). The differences between tumor volume by treatment in the final week of the analysis (week 4) were also not significantly different in either study ($P = 0.07$ and $P = 0.87$ for Studies 1 and 2, respectively) compared with each respective control (Figure 2E and 2F). Individual tumor growth curves can be found in **Supplemental Figure 2**.

Due to the large variability in the *in vivo* tumor volumes, tumor volumes were transformed by their natural logarithms. Regression analysis of *in vivo* log-transformed tumor volumes are shown in **Table 5**. In Study 1, a significant effect of time was noted (b = 0.87, $P < 0.0001$), indicating that the log-transformed tumor volume increased with time (Table 5). The tests for interaction effects indicated that the slopes for TP-Int1 and TP-L were not significantly different from that of the control group (b = −0.04, $P = 0.73$ and b = −0.03, $P = 0.76$, respectively). The main effect of TP-Int1 treatment on log-transformed tumor volume was statistically significant, with a β-coefficient of −0.43 ($P = 0.04$), relative to Con-L. This corresponds to a 35% decrease in the actual tumor volume (mm^3) in TP-Int1 compared with Con-L over the 5-wk interval of tumor growth. The main effect of TP-L on log-transformed tumor volume and Con-L at week 0 was not statistically significant (b = −0.34, $P = 0.11$). Tests of the interaction effects between treatments and time indicated that the slopes for TP-Int1 and TP-L were not significantly different from Con-L (b = −0.04, $P = 0.73$ and b = −0.03, $P = 0.76$, respectively), indicating no differences in tumor growth rates.

Similar to Study 1, a significant effect of time was noted (b = 1.21, $P < 0.0001$) in Study 2, indicating that the mean log-transformed tumor volume increased with time. Likewise, no significant interaction effects between treatment and time were found, indicating that the tumor growth rate for Con-Int did not significantly differ from TP-Int (b = 0.02, $P = 0.79$) or Lyc-Int (b = 0.01, $P = 0.83$). Unlike Study 1, there were no significant main effects of dietary treatment, indicating that Con-Int did not differ from TP-Int2 (b = 0.29, $P = 0.44$) and Lyc-Int (b = 0.35, $P = 0.38$). Sensitivity analyses were conducted for both studies by using a weighted least squares approach and by removing extreme observations. However, the results of these analyses were unchanged.

Discussion

Men undergoing ADT as a component of curative multimodality therapy or for advanced or metastatic disease frequently consume supplements, many containing lycopene or tomato components, or increase their intake of tomato products, in hope of improving therapeutic outcome. There is currently a lack of quality preclinical research or clinical trials supporting the hypothesis that tomato products or lycopene enhance the benefits of therapeutic interventions such as ADT. The present studies address this key gap in the scientific literature using a well-controlled and established TRAMP system with relevant physiological exposure to tomato components and lycopene to quantify their impact on the evolution of CRPC. We hypothesized that dietary tomato or lycopene would reduce CRPC incidence and progression in the TRAMP model based on epidemiological and preclinical data. In contrast to the impact of tomato and lycopene on *de novo* murine prostate carcinogenesis (12–14), we observed no significant impact of these treatments on the primary outcomes in castrated TRAMP mice: incidence of histopathologic cancer, tumor weight at necropsy, final tumor volume by ultrasound, and duration of tumor-free survival (evaluated by ultrasound).

Importantly, this study, like other murine experiments using similar dosages of lycopene or tomato products, resulted in blood concentrations that are relevant to what is observed in humans (13, 14, 20, 28). Although the dose provided in the diet of mice for tomato powder interventions (~3 mg

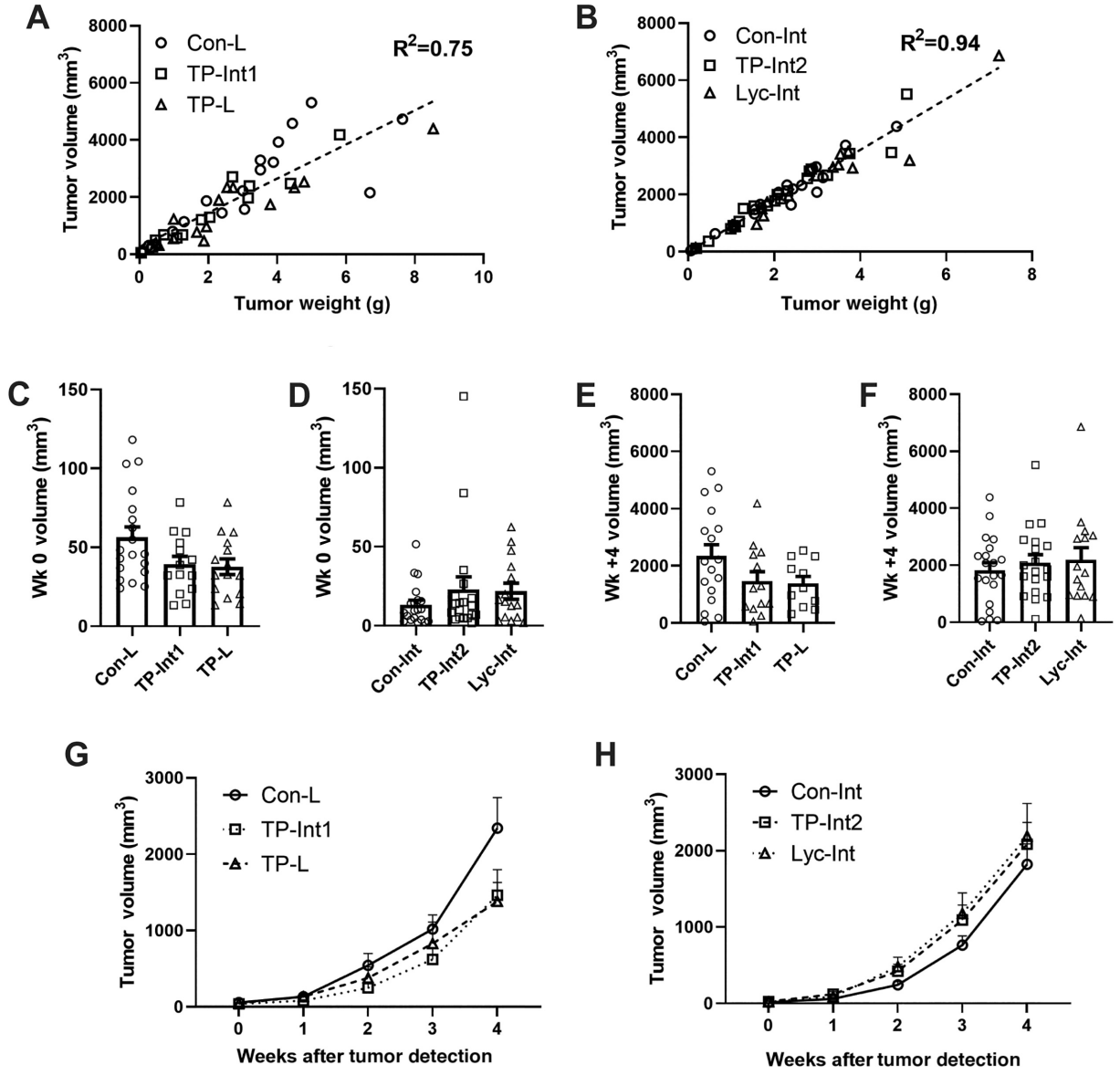

FIGURE 3 Correlation and means of weekly *in vivo* CRPC tumor volumes in TRAMP mice. (A) Study 1 correlation of tumor weight at necropsy compared with tumor volume at final ultrasound scan *in vivo*; (B) Study 2 correlation of tumor weight at necropsy compared with tumor volume at final ultrasound scan *in vivo*; (C) Study 1 *in vivo* tumor volume at tumor detection (week 0); (D) Study 2 *in vivo* tumor volume at tumor detection; (E) Study 1 *in vivo* tumor volume at ultrasound week +4; (F) Study 2 *in vivo* tumor volume at ultrasound week +4; (G) Study 1 weekly ultrasound *in vivo* tumor volumes; (H) Study 2 weekly ultrasound *in vivo* tumor volumes. Data are expressed as means ± SEM. In Study 1, $n = 26$–28 per group (Con-L, $n = 28$; TP-L, $n = 26$; TP-Int1, $n = 26$). In Study 2, $n = 27$–29 per group (Con-Int, $n = 29$; TP-Int2, $n = 29$; Lyc-Int, $n = 27$). For clarity, only the upper error bars are displayed. Individual points represent individual tumors. Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; CRPC, castration-resistant prostate cancer; Lyc-Int, control diet with lycopene beadlets provided after castration; TP-Int1, control diet followed by a tomato powder diet after castration; TP-Int2, a tomato diet with placebo beadlets; TP-L, lifelong consumption of TP; TRAMP, transgenic adenocarcinoma of the mouse prostate.

lycopene/kg body weight) may seem at first glance very excessive or pharmacologic (201 mg of lycopene per day for a 70 kg male), this concentration is necessary in a mouse to achieve blood concentrations similar to humans due to the poor absorption of carotenoids in rodents (29). The ranges of blood lycopene concentrations found in this and similar studies correlates 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 (9, 30, 31). Importantly, this dose is easy to achieve through the diet. A human equivalent dose of 3 mg/kg in mice translates to 17 mg per day (0.24 mg/kg) (32). This could be achieved with a half serving of tomato sauce (1/4 cup, 60 g) per day. Men accumulate carotenoids in the prostate and prostate lycopene concentrations increase similar to blood concentrations (33). This has been demonstrated in studies with the daily intake of standard tomato products such as juice, soup, or sauce over several weeks (34–36). Previous studies have also demonstrated that blood and prostate concentrations of lycopene after consuming tomato juice are related to specific genetic polymorphisms impacting carotenoid absorption and metabolism (37, 38). Together, these studies indicate that the mice in our studies achieve blood and tissue concentrations relevant to humans. As a result, these data are particularly

TABLE 5 Generalized linear mixed model regression analyses of *in vivo* tumor growth

	Estimate[1]	SE	t	*P*
Study 1				
Intercept	3.89	0.14	27.45	<0.0001
Time	0.87	0.07	12.54	<0.0001
Treatment				
TP-L	−0.34	0.21	−1.65	0.105
TP-Int1	−0.43	0.20	−2.13	0.038
Time*Treatment				
TP-L*time	−0.03	0.10	−0.31	0.760
TP-Int1*time	−0.04	0.10	−0.35	0.726
Study 2				
Intercept	2.44	0.27	9.11	<0.0001
Time	1.21	0.05	22.27	<0.0001
Treatment				
TP-Int2	0.29	0.37	0.78	0.440
Lyc-Int	0.35	0.40	0.89	0.380
Time*Treatment				
TP-Int2*time	0.02	0.08	0.26	0.792
Lyc-Int*time	0.02	0.08	0.21	0.832

[1]Estimates and SEs are expressed as: intercept, the natural log of tumor volume (in mm^3) at detection (week 0); time, the natural log of the relative increase in tumor volume in 1 wk in the control group; treatment, the natural log of the relative difference between the *in vivo* tumor volume at week 0 of a respective treatment group and the control group; time*treatment, the natural log of the relative difference between the tumor growth rate of a respective treatment group and the tumor growth rate of the control group. The control group was the reference group in this analysis.

Con-Int, control diet with placebo beadlets; Con-L, lifelong control diet; Lyc-Int, control diet with lycopene beadlets provided after castration; TP, lyophilized tomato paste; TP-Int1, control diet followed by a tomato powder diet after castration; TP-Int2, a tomato diet with placebo beadlets; TP-L, lifelong consumption of TP.

relevant to human dietary interventions and adds confidence to our findings.

Experimental models that closely recapitulate the physiology, molecular biology, and natural selective pressures of CRPC development more reliably estimate the efficacy of preventative or therapeutic strategies. The TRAMP model exhibits castration sensitivity similar to humans, (21) is immunocompetent, exhibits a predictable histological progression from low-grade hyperplasia to poorly differentiated adenocarcinoma (ultimately with clear neuroendocrine features) and local as well as distant metastasis (39–41). Furthermore, transcriptional signatures of human and TRAMP prostate cancer are similar (28, 42). The TRAMP model is also characterized by dysfunction of Rb and p53 due to the SV40 transgene, thereby disrupting cell cycle control and promoting genomic instability; aberrations in TP53 and RB are transcriptional signatures of human CRPC (41). These features of the TRAMP model, both in *de novo* carcinogenesis and in response to castration, fortify our confidence that our new findings are relevant to human castrate-resistant disease. Our data suggests caution in advising males undergoing ADT that change in tomato or lycopene intake will likely impact their disease.

Ultrasound evaluation of the emergence of individual castrate-resistant tumors over 5 wk provided a unique and insightful dimension to our studies. The plots of individual ultrasound-derived tumor volumes (Supplemental Figure 2) displayed extreme heterogeneity in the growth rate of CRPC tumors. This variation was observed regardless of dietary treatment and ranged between 10- and 100-fold. Remarkably, this heterogeneity is similar to the over 10-fold variation that is seen in the rate of progression for men failing initial ADT (43). Although the sample sizes of the present experiments are large compared with other preclinical studies, this observed variation in tumor growth rates makes it difficult to detect modest impacts of the dietary treatments on tumor growth rates. CRPC tumors that grow despite ADT typically maintain activity of androgen-mediated pathways, often through sustained androgen receptor signaling (6). There are many pathways for PCa to progress to CRPC such as mutations affecting the function of the androgen receptor, affinity for alternative ligands, activation of complementary growth-promoting signaling pathways, and others (6, 44). It is likely that the specific mutational spectrum of individual CRPC lesions underlies the large variation in progression rates.

Of the very few studies of PCa progression (45, 46) or CRPC (47–49), none have been sufficiently powered or adequately controlled. A recent systematic review of preclinical studies found that most eligible studies reported inhibitory effects of tomato or lycopene treatment on androgen-related outcomes (50). Lycopene, in addition to other tomato bioactives, may affect tumor progression after castration by modifying inflammatory status (51, 52), androgen and growth factor signaling (28), apoptosis (52–54), and cell cycle progression (52–54). Tomatoes contain other potentially beneficial carotenoids and bioactive compounds that may reduce prostate tumorigenesis (31), and some studies suggest that the whole fruit may be more effective than lycopene alone (14). Further studies are needed that investigate the molecular profiles of CRPC tumors to determine if TP or lycopene feeding differentially impacts specific molecular subtypes of CRPC.

In conclusion, our studies of tomato products in a well-characterized murine model of prostate carcinogenesis are relevant to a key issue for men with PCa undergoing ADT. The emergence and progression of CRPC was not altered by tomato or lycopene consumption. As science progresses, the impact of dietary tomato or lycopene on specific molecular subtypes of CRPC evolution may be explored with the rise of personalized nutrition and cancer treatment plans. Although data from these studies did not display a benefit from tomato consumption following castration (ADT), a recent single-blind, randomized, pilot trial of 32 men on ADT found that adherence to a diet and exercise-based lifestyle intervention shows promise for countering and/or reversing adverse effects of ADT (55). Our findings in a model that is relevant to the evolution of human PCa, with blood concentrations similar to human epidemiological literature, suggest that men should focus on other fitness and healthy dietary guidelines (such as the Dietary Guidelines for America 2015) (56) as they begin ADT rather than focus upon supplements of nutrients or other bioactives until a benefit has been demonstrated by well-designed experimental studies.

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